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(54) Title: HUMAN CD26 AND METHODS FOR USE		
(57) Abstract A polypeptide fragment or analog of CD26 capable of disrupting the naturally-occurring binding interaction between CD45 and CD26, and a method of screening such compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of: a) providing a first and a second sample of cells expressing both CD26 and CD45; b) incubating the first sample in the presence of a candidate compound; c) incubating the second sample in the absence of the candidate compound; d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody; e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.		

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HUMAN CD26 AND METHODS FOR USE

This application is a continuation-in-part of Morimoto et al., USSN 07/832,211. This invention was
5 made at least in part with funds provided under grants from the United States Government (AI 12069, AR 33713). The Government has certain rights in the invention.

Background of the Invention

The field of the invention is human T cell
10 activation antigens.

CD26 is a human T cell activation antigen originally identified by its reactivity with the monoclonal antibody Ta1 (Fox et al., *J. Immunol.* 133:1250, 1984). CD26 has recently been shown to be
15 identical to human dipeptidyl peptidase IV (EC 3.4.14.5) (Ulmer et al., *Scand. J. Immunol.* 31:429, 1990; Barton et al., *J. Leukocyte Biol.* 48:291, 1990). Dipeptidyl peptidase IV (DPPIV) is a serine exopeptidase which is capable of cleaving x-proline or x-alanine (where x is
20 any amino acid) from the amino terminus of certain peptides.

CD26 is recognized by a second monoclonal antibody, anti-1F7 (Morimoto et al., *J. Immunol.* 143:3430, 1989). Dang et al. (*J. Immunol.* 144:4092,
25 1990) report that solid phase-immobilized anti-1F7 mAb is capable of inducing proliferation of human CD4⁺ T lymphocytes in conjunction with submitogenic doses of anti-CD3 or anti-CD2 antibodies. They suggest that the CD26 antigen is involved in CD3- and CD2-induced human
30 CD4⁺ T cell activation.

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Summary of the Invention

In one aspect, the invention features a polypeptide fragment of CD26 lacking amino acid residues 3-9 of the latter sequence. (By "fragment" is meant a portion of CD26 that represents at least 50 consecutive residues of CD26. Such a fragment will preferably represent at least 100 residues of CD26, more preferably at least 200, and most preferably at least 500; it preferably includes the DPPIV active site residues at residues 627-631.) Such a fragment, in which the amino acid residues to the carboxy terminal side of residue 37 are preferably intact, is encoded by the nucleic acid sequence shown as CD26 Δ 3-9 (SEQ ID NO: 2). In preferred embodiments, the polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the product of signal peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

In another aspect, the invention features a polypeptide fragment of CD26 lacking residues 24-34 of the latter sequence. Such a fragment, in which the amino acid residues to the carboxy terminal side of residue 37 are preferably intact, is encoded by the nucleic acid sequence shown as CD26 Δ 24-34 (SEQ ID NO: 3). In preferred embodiments, the polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 3; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the product of signal peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

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In a related aspect, the invention features a plasmid encoding a polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2 (CD Δ 3-9) or 3 (CD Δ 24-34);
5 this plasmid preferably includes an expression control sequence.

Polypeptide fragments of CD26 which are soluble under physiological conditions generally lack most or all of the hydrophobic amino acid residues found near the
10 amino terminus of the polypeptide depicted in SEQ ID NO: 1. This can be accomplished by genetically manipulating a nucleic acid encoding CD26 to delete the hydrophobic residues, or to delete enough of the N-terminal amino acids (e.g., residues 3-9 or 24-34) to leave the
15 resulting polypeptide susceptible to cleavage by signal peptidase. Other fragments of CD26 which are within the invention include those in which all or part of the putative dipeptidyl aminopeptidase catalytic site (Gly₆₂₇ to Gly₆₃₁) is deleted. Such fragments, which include
20 *inter alia* the deletion mutant shown in Fig. 15 (SEQ ID NO: 11); fragments having additional deletions such as those in Δ 3-9 (SEQ ID NO: 2) and Δ 24-34 (SEQ ID NO: 3); and those missing the entire signal peptide region up to Ala₃₅, Thr₃₆, Ala₃₇ or Asp₃₈, would constitute
25 enzymatically inactive fragments of CD26 useful in the screening assays of the invention, as well as for inhibiting complex formation between CD26 and/or CD45 and p43. Along the same lines, a mutant form of CD26 (or a fragment thereof) which lacks DPPIV activity can be
30 generated by replacing one of the residues in the active site with a different amino acid (e.g., by replacing Ser₆₂₉ with Ala).

By "substantially pure" is meant a polypeptide or protein which has been separated from biological
35 macromolecules, (e.g., other proteins, carbohydrates,

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etc.) with which it naturally occurs. Typically, a protein or polypeptide of interest is substantially pure when less than 25% (preferably less than 15%) of the dry weight of the sample consists of such other

5 macromolecules.

By "physiological conditions" is meant an aqueous solution, whether *in vivo* or *in vitro*, having a pH and salt concentration similar to that found in serum.

Phosphate buffered saline is an example of a commonly
10 used buffer in which a polypeptide that is soluble under physiological conditions would be soluble.

By "substantially identical to CD26" is meant that at least 80%, preferably at least 90%, more preferably at least 95%, most preferably at least 99%, of the amino
15 acid sequence is identical to that of the corresponding portion of CD26, and any non-identical amino acids in the sequence are amino acid substitutions, preferably conservative, which do not eliminate the biological activity of the molecule.

20 By "plasmid" is meant an extrachromosomal DNA molecule which includes sequences that permit replication within a particular host cell.

By "expression control sequence" is meant a nucleotide sequence which includes recognition sequences
25 for factors that control expression of a protein coding sequence to which it is operably linked. Accordingly, an expression control sequence generally includes sequences for controlling both transcription and translation: for example, promoters, ribosome binding sites, repressor
30 binding sites, and activator binding sites.

In another aspect, the invention features a polypeptide fragment of CD26 capable of disrupting the naturally-occurring binding interaction between CD45 and CD26. Polypeptides which disrupt the interaction between

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CD26 and CD45 can be identified, for example, using the immunoprecipitation assay described below.

In another aspect, the invention features a method for screening candidate compounds to identify compounds
5 capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of
10 a candidate compound;

(c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26
15 antibody;

(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD45 present
20 in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the
25 binding.

As used herein, an anti-CD26 antibody is one capable of forming a specific immune complex with CD26, i.e., the antibody binds directly to CD26 but does not substantially bind directly to other molecules in the
30 assay of the invention.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:

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(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

5 (c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD45 antibody;

10 (e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD26 present in the first immunoprecipitate is less than the amount of
15 CD26 present in the second immunoprecipitate, the presence of a lesser amount of CD26 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

20 In another aspect, the invention features a monoclonal antibody which, when contacted under physiological conditions with a cell (preferably a eukaryotic cell such as a mammalian cell) expressing CD26 and CD45, interferes with the association of CD26 and
25 CD45; and a method for assaying for such an antibody.

In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD45 on its surface; and

30 (b) introducing into the cell a nucleic acid encoding CD26, such that the cell expresses CD26 on its surface.

In yet another aspect, the invention features a method which includes:

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(a) providing a cell which expresses CD26 on its surface; and

(b) introducing into the cell a nucleic acid encoding CD45, such that the cell expresses CD45 on its
5 surface.

In other aspects, the invention includes a cell transfected with a nucleic acid encoding CD26, the cell expressing both CD26 and CD45 on its surface; and a cell transfected with a nucleic acid encoding CD45, the cell
10 expressing both CD26 and CD45 on its surface. In preferred embodiments, the cells are T-cells such as Jurkat cells.

In another aspect, the invention features a method which includes:

15 (a) providing a cell which expresses neither CD26 nor CD45 on its surface; and

(b) transfecting the cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

In yet another aspect, the invention includes a
20 method of generating a hybridoma cell, which method includes:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

25 (b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line (i.e., a line of cells which can be maintained indefinitely in culture) to
30 produce a hybridoma cell.

In a related aspect, the invention features a hybridoma cell generated by:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its
35 surface;

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(b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line to produce a hybridoma cell, wherein the hybridoma cell produces a monoclonal antibody specific for CD26. Applicable methods of inducing an immune response in an animal by using cells as the antigen, and fusing B lymphocytes with immortal cells to produce hybridoma cells, are well known to those of ordinary skill in the art of making hybridomas. The resulting hybridomas are then cloned and screened for production of monoclonal antibodies which bind to cells expressing the CD26 antigen, but not to identical cells which do not express the CD26 antigen.

Also within the invention are cell-free preparations of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof. Such complexes may be conveniently prepared by recombinant expression of each of the relevant polypeptides in a manner that prevents their being anchored to the cellular membrane (e.g., by use of a soluble fragment of each), or by isolation of the full-length proteins from a cell membrane preparation, and by combining the two polypeptides to form the desired complex either before or after removal of contaminating cellular constituents. Such complexes would be useful, e.g., for generating monoclonal antibodies specific for the complex, and for screening for compounds capable of interfering with the association of CD26 and CD45.

Also within the invention are purified preparations of p43, a 43 kDa molecule which, like CD45, associates with CD26 in cells and therefore is thought to play a role in T cell activation, and cell-free preparations of CD26 (or a fragment thereof) complexed with p43 (or a fragment thereof). The screening assay

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described above for compounds capable of inhibiting the interaction of CD26 and CD45 can be readily adapted to detect compounds (including fragments of CD26 or p43) capable of inhibiting the interaction of CD26 and p43.

- 5 Also within the invention is a therapeutic composition containing a fragment of CD26 (e.g., water-soluble CD26), in a pharmaceutically acceptable carrier (e.g., saline or any aqueous or nonaqueous substance which is suitable for injection), or intact CD26
- 10 incorporated into a liposomal preparation or other carrier substance suitable for a polypeptide such as CD26. Such a therapeutic composition can be used in a method for modulating the immune response of a patient (e.g., enhancing the immune response of an
- 15 immunosuppressed patient) by administering the composition by any appropriate means to the patient. It is expected to be particularly useful for the treatment of immunosuppression in a patient infected with human immunodeficiency virus (HIV) and having AIDS or AIDS-
- 20 related complex, but may also be used where the patient's immune system is depressed as a result of treatment with an immunosuppressive compound, or acquired immunodeficiency of undetermined etiology, or congenital immunodeficiency.
- 25 The compounds of the invention are, when combined with a pharmaceutically acceptable carrier, also useful as vaccine adjuvants, to be administered to an individual vaccinee in conjunction with (i.e., immediately before, after, or along with) a vaccine antigen in order to
- 30 enhance the immune response produced by such antigen. Examples of vaccine antigens which may be used with the adjuvant of the invention include those containing chemically inactivated or genetically engineered viral or bacterial products, such as diphtheria or pertussin

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toxoid or recombinant viral proteins, and those containing live but attenuated virus or bacteria.

The assays described herein may be used to screen candidate immunosuppressive compounds by a method including the steps of (a) contacting a lymphocyte with CD26 or a fragment of CD26 in the presence of a candidate compound, and (b) determining whether the candidate compound inhibits the CD26-induced proliferation of the lymphocyte, such inhibition being an indication that the candidate compound has immunosuppressive activity. The assays may instead be used to screen CD26 fragments for immunostimulatory activity. One such assay would include the following steps: (a) contacting a lymphocyte with a candidate CD26 fragment, and (b) determining whether the fragment increases the rate of proliferation of the lymphocyte, such increase being an indication that the fragment has immunostimulatory activity. Alternatively, one could simply assay the fragment for dipeptidyl peptidase IV activity, such activity being an indication that the fragment has immunostimulatory activity.

Also within the invention is a solid matrix material (e.g. Affi-Gel™ (Bio-rad)) to which CD26 or a fragment thereof is attached.

CD26 is known to play a role in T cell activation. By interfering with the normal functioning of CD26, one can control the process of T cell activation, and thus prevent such unwanted immune responses as transplant rejection and certain autoimmune diseases. The information disclosed herein concerning proteins with which CD26 associates on the T cell provides the means for designing and screening compounds that interfere with CD26 function in the cell.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

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Detailed Description

The drawings are first briefly described.

Drawings

Fig. 1 depicts the nucleotide sequence and deduced amino acid sequence (SEQ ID NO:1) of the cDNA clone for human CD26.

Fig. 2 depicts the results of an indirect fluorescence staining assay.

Fig. 3 is a pair of photographs of gels illustrating the results of immunoprecipitation analysis (panel A) and enzymatic activity analysis (panel B).

Fig. 4 is a set of graphs depicting the results of a $[Ca^{2+}]_i$ mobilization assay.

Fig. 5 is a graph illustrating the effect of various treatments on interleukin-2 production.

Fig. 6 is a photograph of a gel illustrating the results of immunoblotting analysis.

Fig. 7 depicts the results of FACS analysis.

Figs. 8-12 are photographs of gels illustrating the results of immunoprecipitation assays.

Fig. 13 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of $\Delta 3-9$ (SEQ ID NO: 2) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

Fig. 14 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of $\Delta 24-34$ (SEQ ID NO: 3) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

Fig. 15 depicts the amino acid sequence of a CD26 fragment lacking a portion of the carboxy terminal region of CD26 (SEQ ID NO: 11).

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Fig. 16 is a graph illustrating the effect of soluble CD26, soluble CD45, and soluble CD4 on PBL proliferation.

Sequencing and Characterization of CD26

- 5 Described below is the cloning and sequencing of a full-length CD26 cDNA. Also described are a series of experiments which demonstrate that: (1) modulation of CD26 from the surface of T lymphocytes leads to enhanced CD3 ζ phosphorylation and increased CD4-associated p56^{lck}
10 tyrosine kinase activity; (2) CD26 is comodulated with CD45; and (3) CD26 and CD45 are closely associated.

Cells and Antibodies

- Human peripheral blood mononuclear cells (PBMC), E rosette-positive cells and PHA-activated T cells for
15 use in the experiments described below were prepared as follows. Human PBMC were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (LKB Biotechnology, Inc., Piscataway, NJ). Unfractionated mononuclear cells were separated into E
20 rosette-positive (E+) and E rosette-negative (E-) populations, and the E+ cells were depleted of contaminating monocytes as described (Morimoto et al., *J. Immunol.* 134:3762, 1985; Morimoto et al., *J. Immunol.* 134:1508, 1985; Matsuyama et al., *J. Exp. Med.* 170:1133,
25 1989). These T cells were used for experiments involving T cells in this report. E+ cells were stimulated with PHA (0.25 μ g/ml) and rIL-2 (40 U/ml) for 7 days in RPMI 1640 medium supplemented with 10% human AB serum, 4mM L-glutamine, 25 mM HEPES buffer, 0.5% sodium bicarbonate,
30 and 1% penicillin/streptomycin (culture medium) and used as PHA blasts. The monoclonal antibodies used were anti-CD26 (Ta1/4EL-1C7, IgG₁; 1F7, IgG₁; 5F8, IgG₁), and anti-CD3 (T3/RW24B6; IgG_{2b}) (Fox et al., *J. Immunol.* 133:1250, 1984; Morimoto et al., *J. Immunol.* 143:3430, 1989;
35 Morimoto et al., *J. Immunol.* 134:3762, 1985). Anti-CD29

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(4B4; IgG₁) (Morimoto et al., *J. Immunol.* 134:3762, 1985) was used as an isotype-matched control throughout.

Isolation of a CD26 cDNA

- To isolate a CD26 cDNA, a cDNA library was
- 5 constructed from mRNA isolated from human PHA-activated T cells using the CDM7 vector. Briefly, poly(A)+ RNA was prepared from 4-day-old PHA-activated T cells by the guanidinium isothiocyanate method (Chirgwin et al., *Biochem.* 18:5294, 1979), and an expression library was
- 10 prepared as previously described, except that the vector CDM7, a precursor to CDM8 lacking polyoma sequences, was employed (Aruffo et al., *Proc. Natl. Acad. Sci. USA* 84:8573, 1987; Seral et al., *Proc. Natl. Acad. Sci. USA* 87:3365, 1987). Recombinant hybrid plasmids were
- 15 transfected into COS cells, and CD26 expressing cells were immunoselected with the monoclonal antibody, anti-Ta1 (Aruffo et al., *supra*; Seed et al., *supra*). Reactive cells were retained on antibody coated dishes, and plasmids were recovered from transfected cells. Plasmid
- 20 DNAs were further selected by three additional rounds of transfection and immunoselection. Two of eight clones thus isolated were found to encode anti-Ta1 reactive determinants. The two clones were identical by restriction enzyme fragment mapping.
- 25 Sequencing of both strands of the isolated 2.9 kb CD26 cDNA by the dideoxy sequencing method revealed a 2298 base pair open reading frame beginning with an ATG at nucleotide 11 which conforms to consensus translational initiation sites (Fig. 1). The deduced
- 30 CD26 structure is a 766 amino acid residue polypeptide with a molecular weight of approximately 88,300 (SEQ ID NO: 1).

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Predicted Structure of CD26

The predicted CD26 polypeptide has a single stretch of hydrophobic amino acids in the N-terminal region between residues 7 and 28 (Fig. 1, boxed), which
5 is sufficiently long and hydrophobic to span a lipid bilayer (Davis et al., *Cell* 41:607, 1985). The sequence is preceded by six N-terminal residues which contain polar and charged residues, and is followed by charged residues that would not allow cleavage by signal
10 peptidase (von Heijne, *Nucl. Acids Res.* 14:4683, 1986). This sequence thus has the characteristics of a signal sequence of a type II membrane protein, which serves both to direct the translocation of the nascent protein across the membrane of the rough endoplasmic reticulum, and to
15 anchor the mature protein in the membrane (Hong et al., *supra*, 1990; Shipp et al., *Proc. Natl. Acad. Sci. USA* 85:4819, 1988; Thomas et al., *J. Clin. Invest.* 83:1299, 1989). Furthermore, the fact that potential N-glycosylation sites are located in the carboxy side of
20 the hydrophobic core (Fig. 1, short underlines) suggests that CD26 is a type II membrane protein. Therefore, the N-terminal 6 amino acid residues are predicted to be cytoplasmic, and the next 22 amino acids, which are primarily hydrophobic, are predicted to transverse the
25 cytoplasmic membrane. The 738 C-terminal amino acids constitute the predicted extracellular domain of CD26.

The predicted extracellular domain of CD26 may be conveniently divided into three regions: an N-terminal glycosylated region (residues 29 to 323), a relatively
30 cysteine-rich middle section (residues 324 to 551), and a C-terminal region (residues 552 to 766) (Fig. 1). The N-terminal region contains 8 of the 10 potential attachment sites for N-linked glycans (Fig. 1, short underlines) (Marshall, *Ann. Rev. Biochem.* 41:673, 1972), and one of
35 the 12 cysteine residues (Fig. 1, asterisks). In

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contrast, the subsequent cysteine-rich section contains 9 cysteines but only one N-linked glycosylation site. The C-terminal region contains two cysteines, one N-linked glycosylation site and a potential catalytic site (Fig. 1, double underline), the sequence G-W-S-Y-G at position 627 to 631. This sequence fits the consensus G-X-S-X-G found in the active sites of serine proteases and esterases, although tryptophan and tyrosine flanking the catalytic serine are unusual residues at these positions (Brenner, *Nature* 334:528, 1988).

Homology with the Other Proteins.

The predicted amino acid sequence of the human CD26 antigen (SEQ ID NO: 1) is 85% homologous to the deduced rat DPPIV enzyme sequence predicted from cDNAs isolated from rat liver and kidney libraries. Considering this high degree of homology and the fact that anti-Ta1 antibody reacts with human liver and kidney epithelium (Mobius et al., *Exp. Immunol.* 74:431, 1988), the DPPIV enzyme present in those tissues is probably the functional counterpart of the CD26 antigen. This high degree of homology also supports the prediction of the membrane topology of CD26, because rat DPPIV has been shown to be a type II membrane protein (Hong et al., *supra* 1990).

Aside from the signal sequence, the greatest homology between rat (Ogata et al., *supra*) and human CD26/DPPIV proteins is in the C-terminal region, which includes the putative catalytic site. In fact, the sequences are identical from residues 624 to 724, and 94% homologous from residues 552 to 766. This C-terminal region is 46% homologous to a region of the predicted yeast aminopeptidase B (DPAPB) sequence (Roberts et al., *J. Cell. Biol.* 108:1363, 1989). Further, CD26 amino acid residues 107 to 233 are 36% homologous to DPAPB. The yeast DPAPB enzyme is also a type II membrane dipeptidyl

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aminopeptidase, and is involved in the maturation of the yeast pheromone alpha factor. The putative catalytic sequence G-W-S-Y-G is conserved between human and rat CD26/DPPIV and yeast DPAPB.

- 5 Recently the structures for CD10 and CD13 were determined by cDNA cloning (Shipp et al., *supra*, Thomas et al., *supra*). These antigens are ectoenzymes which have neutral endopeptidase [EC. 3.4.24.11] and aminopeptidase N [EC. 3.4.11.2] activities, respectively.
- 10 Although CD10 and CD13 are also type II membrane proteins, there is no significant sequence homology between these enzymes and CD26.

 Although the CD26 antigen is known to be a functional collagen receptor (Dang et al., *J. Exp. Med.* 172:649, 1990), a homology search did not find significant homology with any other known collagen-binding proteins such as fibronectin, CD11b and the integrins.

Characterization of CD26 Antigen expressed on Transfected
20 Jurkat Cells

- To characterize the cDNA-encoded CD26 antigen, the human T cell leukemia line, Jurkat, was transfected with the expression plasmid pSR α 26, in which the CD26 cDNA was placed under the control of the SR α promoter. Briefly,
- 25 the CD26 cDNA insert was cloned into the PstI and EcoRI sites of the plasmid pCDLSR α 296 (Takebe et al., *Mol. Cell. Biol.* 8:466, 1988) by blunt-end ligation to create the CD26 expression plasmid, pSR α -26. pSR α -26, digested with SalI, and pSV2neo-SP (confers neomycin resistance to
- 30 host cells; Streuli et al., *EMBO J.* 8:787, 1989), digested with PvuI, were used to co-transfect Jurkat cells according to Streuli et al. (*supra*). Transfectants were initially selected in RPMI1640 supplemented with 10% fetal calf serum, 4mM glutamine and 1.0 mg/ml Geneticin
- 35 (Gibco/BRL, Bethesda, MD). Subsequently, the

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concentration of Geneticin was gradually decreased to 0.25 mg/ml during the selection period. Geneticin-resistant clones were further screened for CD3 and CD26 antigen expression by cell-surface staining as described 5 below. Transfectants were maintained in the above medium containing 0.25 mg/ml Geneticin.

Staining of cell surface antigens with monoclonal antibodies and flow cytometry analyses using an EPICS V cell sorter (Coulter) were performed as described by Dang 10 et al. (*J. Immunol.* 144:4092, 1990).

Parental Jurkat cells do not express detectable amounts of the CD26 antigen as determined by cell surface staining (Fig. 2), or by a binding assay with radiolabeled Ta1 monoclonal antibody. Northern blotting 15 analysis revealed that this cell line also does not express CD26 mRNA even after phorbol 12-myristate 13-acetate (PMA) treatment, which is known to induce CD26 expression (Dang et al., *J. Immunol.* 145:3963, 1990). Referring to Fig. 2, the Jurkat-CD26 transfectant 26.C28 20 had high expression of the CD26 antigen. On the other hand, another Jurkat-CD26 clone, 26.24, expressed only moderate levels of the antigen. Both transfectants were reactive with three anti-CD26 monoclonal antibodies (Ta1, 1F7, and 5F8) which define three distinct CD26 antigen 25 epitopes.

To study whether the CD26 antigen expressed on Jurkat T cell lines had the same characteristics as that on peripheral blood lymphocytes, immunoprecipitation experiments were carried out.

30 Briefly, cell surface proteins were labelled with lactoperoxidase-catalyzed iodination as described by Morimoto et al., (*J. Immunol.* 143:3430, 1989). Immunoprecipitations (employing an NP-40 lysis buffer) using 1F7 monoclonal antibody were performed as described 35 by Morimoto et al. (*supra*, 1989). Immunoprecipitated

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proteins were separated by 8% SDS-PAGE under reducing conditions.

Referring to Fig. 3 (panel A), 1F7 monoclonal antibody immunoprecipitated a 110 kDa protein from the CD26 transfected Jurkat cells (lanes 2 and 3) as well as from PHA blasts (lane 4). There was no detectable 110 kDa band in nontransfected (lane 1) and vector-only transfected Jurkat cells. Control anti-4B4 monoclonal antibody immunoprecipitated a comparable amount of 130 kDa protein from each of the cell lines. Interestingly, 1F7 immunoprecipitated an additional 43 kDa protein from both transfectants and PHA blasts. Similar results were observed using peripheral blood T cells. This 43 kDa protein may contribute to T cell activation through its association with CD26.

DPPIV enzymatic activity was measured using an Enzyme Overlay Membrane system (EOM, Enzyme System Products, Dublin, CA). Briefly, lysates were incubated with SDS sample buffer for 1 hr at room temperature and separated by SDS-PAGE under non-reducing conditions. Following electrophoresis, the EOM moistened with 0.5M Tris-HCl, pH 7.8, was placed on the surface of the gel and this sandwich was incubated for 20 min in a humidified box at 37°C. The reaction was monitored by long wavelength ultraviolet light. Referring to Fig. 3, panel B, DPPIV enzymatic activity was associated with a 160 kDa protein in both transfectants (lanes 2 and 3) and PHA blasts (lane 4), but not in parental Jurkat cells (lane 1), or vector-only transfected cells. It should be noted that the DPPIV enzyme activity was stable in both non-reducing and reducing conditions but disappeared after boiling of the samples. While the apparent molecular weight of CD26 was 160,000 for preparations that were not boiled prior to electrophoresis, the molecular weight of CD26 antigen was 110,000 if the

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protein was boiled prior to SDS-PAGE analysis. Similar results have been reported for rat hepatocyte DPPIV (Walburg et al., *Exp. Cell. Res.* 158:509, 1985). Taken together, the above-described results indicate that the

5 CD26 antigen expressed on the transfected Jurkat cells was the same as that on peripheral blood T cells.

Functional Analysis of CD26 Antigen on Jurkat Transfectants

To determine whether the CD26 antigen expressed on

10 transfected Jurkat cells has biological activity similar to that of CD26 expressed on peripheral blood T cells, we examined $[Ca^{2+}]_i$ mobilization induced by CD26 antigen triggering.

Briefly, loading of indo-1 pentaacetoxymethyl

15 ester (Calbiochem, San Diego, CA) into cells and the measurement of its fluorescence by flow cytometry were performed as described by (Blue et al., *J. Immunol.* 140:376, 1988). Indo-1-loaded cells were preincubated for 1-2 minutes with antibodies and the basal

20 intracellular calcium levels were determined for 33 seconds before the addition of polyclonal goat anti-mouse antibody (10 μ g/ml) (Tago, Burlingame, CA). The RW24B6 anti-CD3 antibody was titrated in this system to determine the submitogenic dose for triggering each cell

25 type. After preincubation of each transfectant with anti-CD26 and/or a submitogenic dose of anti-CD3, anti-mouse antibody was added (time point of addition indicated by small arrows in Fig. 4). Antibody concentrations were 1 μ g/ml for anti-1F7 and 20 ng/ml for

30 anti-CD3.

Referring to Fig. 4, crosslinking of anti-CD26 and submitogenic doses of anti-CD3 with goat anti-mouse immunoglobulin on CD26 transfectants resulted in greater $[Ca^{2+}]_i$ mobilization than crosslinking of anti-CD3 alone.

35 These antibodies did not induce $[Ca^{2+}]_i$ mobilization

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without cross-linking. It is well known that the $[Ca^{2+}]_i$ mobilization signal is divided into two phases: the initial transient rise, and the sustained increase phase (Gardner, *Cell* 59:15, 1989; Goldsmith et al., *Science* 240:1029, 1988). For both CD26 transfectants, the anti-CD26 and anti-CD3 crosslinking induced a strong initial $[Ca^{2+}]_i$ increase (Fig. 4). In addition, for the clone 26.C28, crosslinking induced a sustained increase of the $[Ca^{2+}]_i$ level as well (Fig. 4). The differential pattern of $[Ca^{2+}]_i$ mobilization of the two transfectants may be attributed to the difference in the amount of CD26 antigen expressed by these two transfectants. The enhanced $[Ca^{2+}]_i$ mobilization was specific because, as was reported for peripheral blood T cells (Dang et al., *J. Immunol.* 145:3963, 1990), crosslinking of the CD26 antigen alone did not induce $[Ca^{2+}]_i$ mobilization. Furthermore, crosslinking of anti-CD26 and anti-CD3 did not enhance the $[Ca^{2+}]_i$ mobilization of nontransfected or vector-only transfected Jurkat cells, and crosslinking of the isotype-matched control antibody, anti-4B4, did not result in enhanced $[Ca^{2+}]_i$ mobilization of the transfectants. Similar to the data observed with transfectants, a small but significant transient rise in $[Ca^{2+}]_i$ mobilization was observed in normal resting T cells following CD26 and CD3 crosslinking.

IL-2 production by transfected cells cultured in antibody-coated plates was measured as described by Dang et al., *J. Immunol.* 144:4092, 1990), except that the cell concentration was adjusted to 2×10^6 cell/ml. After 24 hr of culture, supernatants were assayed for IL-2 production using ELISA (R&D system, Minneapolis, MN). Referring to Fig. 5, incubation of the clone 26.C28 transfectants with solid-phase-immobilized anti-1F7 and anti-CD3, which mimicked the crosslinking by anti-mouse antibody, induced the production of a significant amount of IL-2 (striped

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bar), as compared to the control, vector-only transfected, Jurkat cells (solid bar). These results indicate that the CD26 Jurkat transfectants were functionally similar to peripheral blood T cells.

- 5 Moreover, the above data indicate that the stimulatory effect of anti-CD26 and anti-CD3 crosslinking in T cells was in part mediated by an enhancement of $[Ca^{2+}]_i$ mobilization. Since it is well known that the transient rise, as well as the sustained increase, in $[Ca^{2+}]_i$ is
10 necessary for IL-2 production (Gardner, *supra*; Goldsmith, *supra*), the sustained increase of the $[Ca^{2+}]_i$ observed in clone 26.C28 may be the basis for enhanced IL-2 production seen with the transfectant following anti-CD26 and anti-CD3 stimulation. Thus, the data obtained using
15 Jurkat CD26 transfectants provide direct evidence that the CD26 antigen plays an integral role in T cell activation.

Co-association of CD26 and CD45

- The experiments described below demonstrate that
20 modulation of CD26 on the surface of T lymphocytes by anti-CD26 monoclonal antibody leads to enhanced phosphorylation of CD3 and increased p56^{lck} tyrosine kinase activity. Modulation experiments described below demonstrate that CD26 is co-modulated with CD45.
25 Finally, immunoprecipitation assays described below demonstrate that CD26 and CD45 are closely associated. Taken together, the results indicate that an interaction between CD26 and CD45 increases p56^{lck} tyrosine kinase activity, CD3 chain phosphorylation, and T lymphocyte
30 activation.

Enhancement of CD3 Phosphorylation Following anti-CD26 (1F7) Treatment

To evaluate the effect of anti-CD26 antibodies on one of the earliest signaling events in T cell

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activation, we investigated their role in the tyrosine phosphorylation of CD3 ζ .

Immunoblotting analysis of tyrosine phosphorylation of CD3 ζ was performed as described by Vivier et al. (*J. Immunol.* 146:206, 1990). Briefly, peripheral blood T cells (10×10^6 per sample) were incubated in culture media alone or with anti-CD26 (1F7; 1:100 ascites dilution) for various times at 37°C. Cells were then extensively washed in ice cold PBS containing

5mM EDTA, 10mM NaF, 10mM sodium pyrophosphate, and 0.4mM sodium vanadate, then solubilized in lysis buffer (1% NP-40, 150mM NaCl, 50mM Tris HCl, pH 8.0, 5mM EDTA, 1mM PMSF, 10mM iodoacetamide, 10mM NaF, 10mM sodium pyrophosphate, 0.4mM sodium vanadate) for 15 min on ice.

After removing insoluble material by centrifugation at 12,000 rpm for 15 min, samples were combined with an equal volume of sample buffer (2% SDS, 10% glycerol, 0.1M Tris [pH 6.8] 0.02% bromophenol blue), reduced with 5% 2-mercaptoethanol, and separated on 12% SDS-polyacrylamide gels. After separation on SDS-PAGE, cell lysates were transferred to nitrocellulose, and developed using ^{125}I -labelled anti-phosphotyrosine (UBI, NY; 100,000 cpm/ml in PBS containing 1% BSA). Affinity-purified anti-phosphotyrosine was iodinated to a specific radioactivity of 10-20 $\mu\text{Ci}/\mu\text{g}$ protein using iodobeads (Pierce Chemical Co., Rockford, IL).

Referring to Fig. 6, a 21 kD tyrosine phosphoprotein (p21), which has been previously identified in T cells stimulated with various stimuli as phosphorylated CD3 ζ (Vivier et al., *supra*, 1990; Vivier et al., *J. Immunol.* 146:1142, 1991; Ashwell et al., *Annu. Rev. Immunol.* 8:139, 1990), was detected at a constitutive level in samples not treated with anti-CD26 (lane 1). Anti-CD26 treatment significantly increased the phosphorylation of CD3 ζ over the constitutive level

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after 1 hour of anti-CD26 incubation (lane 2). The level of phosphorylated CD3 ζ gradually increased with time, reaching a maximum level after 4 hours of anti-CD26 incubation (lanes 3 and 4; 2 and 4 hours of anti-CD26 treatment respectively), and gradually decreased upon longer incubation (lanes 5 and 6; 6 and 8 hours of anti-CD26 treatment respectively). The total amount of CD3 ζ chain (phosphorylated and non-phosphorylated) present, determined by immunoblotting the same membrane with an anti-CD3 ζ mAb, was similar in all samples. Although anti-CD26 by itself can not induce T cell proliferation, these results show that CD26 modulation provides an initial T cell activation signal as measured by enhanced CD3 ζ phosphorylation.

15 Comodulation of CD26 and CD45 by anti-CD26 Antibody (1F7) Treatment

The fact that the cytoplasmic domain of CD26 (DPPIV) in the rat includes only six amino acid residues suggests that CD26 might be associated with another molecule which acts in a signal transducing capacity, as has been found in the case of the IL-6 receptor and the IL-2 (p55) receptor (Taga et al., *Cell* 58:573, 1989; Robb et al.; *J. Exp. Med.* 165:1201, 1987). The experiments described below indicate that CD26 is associated with another cell surface molecule, CD45. Human peripheral blood T cells were used in the experiments described below and obtained as described by Dang et al. *J. Immunol.* 144:4092, 1990. Anti-CD26 (1F7) induced modulation was performed as previously described (by Dang et al. *J. Immunol.* 145:3963, 1990). Briefly, peripheral blood T cells were incubated overnight at 37°C in medium containing anti-CD26 (1F7) at 1:100 ascites dilution. Cells were then collected, washed and stained with anti-CD26 (1F7) and FITC-conjugated goat anti-mouse IgG; or they were stained with anti-CD45RA (2H4)-PE, anti-CD2-PE,

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anti-CD3-PE (Coulter) or biotinylated anti-CD45RO (UCHL-1) and PE-conjugated avidin.

Flow cytometry analysis was performed using an Epics V cell sorter (Coulter Electronics) as previously described (Morimoto et al., *J. Immunol.* 143:3430, 1989).

The negative control of each fluorescence was less than 5%. The FACS analysis presented in Fig. 7 are representative of three separate experiments. As shown in Fig. 7, overnight incubation with anti-CD26 led to a significant reduction in CD26 expression on T cells.

Interestingly, while CD26 modulation did not have any detectable effect on CD2, CD3 or CD45RA expression, the expression of CD45RO, particularly the high fluorescence peak of CD45RO, was markedly reduced. In addition, modulation of CD2, CD3, or CD4 with respective antibodies had no effect on CD45RO expression. Thus, the co-modulation of CD45RO induced by anti-CD26 treatment appears to be specific for this structure.

Co-immunoprecipitation of CD26 with CD45

The immunoprecipitation experiments described below provide evidence of a direct association between CD26 and CD45. Peripheral blood T cells (50×10^6) were labeled at the surface by lactoperoxidase-catalyzed iodination and immunoprecipitated from NP-40 lysis buffer (0.5% NP-40, 140mM NaCl, 1mM PMSF, 5mM EDTA, 50mM Tris HCl [pH 7.4]) or digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 150mM NaCl, 1mM PMSF, 20mM Triethanolamine [pH 7.8]) using anti-CD26 (Ta1, Coulter Immunology, Hialeah, FL; or 1F7, Dr. C. Morimoto, Dana-Farber Cancer Institute, Boston, MA) and anti-CD45 (GAP 8.3, Berger et al., *Human Immunol.* 3:231, 1981) as previously described by Morimoto et al. (*J. Immunol.* 143:3430, 1989) and Anderson et al. (*Nature* 341:159, 1989). All samples were analyzed under reducing conditions.

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For immunodepletion studies, peripheral blood T cells were labeled and lysed in digitonin lysis buffer as described above. The lysates were precleared by four successive immunoprecipitations with anti-CD45 (GAP 8.3, American Type Culture Collection, Bethesda, MD) or anti-CD1 (T6) and then precipitated by anti-CD26 and anti-CD45.

Digestion with V8 protease from *S. aureus* was carried out during gel electrophoresis as described by Cleveland et al. (*J. Biol. Chem.* 252:1102, 1977). After the first gel electrophoresis, gel slices containing the high molecular weight proteins co-precipitated with CD26 and CD45 proteins were excised and polymerized into the stacking gel of a 15% SDS-polyacrylamide gel. 2.5 μ g of V8 protease in 10 μ l of sample buffer (0.1% SDS, 0.125M Tris-HCl [pH 6.8], 10% glycerol, 0.1% bromophenol blue) were added to wells above the polymerized gel slices. Gel electrophoresis was carried out uninterrupted for 12 hours.

Fig. 8 presents the results of immunoprecipitation analysis without prior depletion. Surface labeled T-lymphocytes were solubilized in NP-40 (lanes 1-4) or digitonin (lanes 5-8) and immunoprecipitated with anti-CD1 (T6) as a negative control (lanes 1 and 5); anti-CD26 (1F7, lanes 2 and 6); anti-CD26 (Ta1, lanes 3 and 7); or anti-CD45 (GAP 8.3, lanes 4 and 8).

While anti-CD26 (Ta1 and 1F7) antibodies precipitated a 110KD molecule from NP-40 lysates under reducing conditions, in digitonin lysates these same antibodies precipitated two major proteins at 180 and 190kD and minor bands at 205 and 220kD in addition to the 110KD band. These additional bands display similar mobility to the CD45 control immunoprecipitates. In this regard, utilizing digitonin lysates or chemical cross-linkers, others have found an association of CD45 with

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Thy-1, CD3, and CD2 (Volarevic et al., *Proc. Natl. Acad. Sci. USA* 87:7085, 1990; Schraven et al., *Nature* 345:71, 1990).

To provide further evidence that the high molecular weight structure which co-precipitated with CD26 is CD45, we carried out both sequential immunodepletion and one-dimensional peptide mapping studies using V8 protease.

Fig. 9 presents the results of immunoprecipitation analysis of samples previously depleted for CD45 using anti-CD45 antibody (GAP 8.3, lanes 4-6) or, as a control, CD-1 using anti-CD1 antibody (T6, lanes 1-3). After depletion, anti-CD26 (1F7, lanes 1 and 4), anti-CD26 (Ta1, lanes 2 and 5), or anti-CD45 (GAP 8.3, lanes 3 and 6) was used for immunoprecipitation. As can be seen in Fig. 9, depletion of CD45 resulted in a complete loss of the high molecular weight structures in the CD26 immunoprecipitate (lanes 4, 5). Furthermore, V8 protease-dependent digestion of the high molecular weight molecules co-precipitated with either CD26 and CD45 yielded identical peptide patterns (Fig. 10). Although CD26 comodulated only with CD45RO (the 180kD isoform), the immunoprecipitation experiments suggest that CD26 is also associated with the 190kD isoform of CD45, and to a lesser degree, with the 205 and 220kD isoforms as well. These observations are consistent with earlier studies demonstrating that CD26 was preferentially expressed on CD45RO+ helper T cells, which are known to preferentially express both the 180 and 190kD isoforms of CD45 (Morimoto et al., *J. Immunol.* 143:3430, 1989; Rudd et al., *J. Exp. Med.* 166:1758, 1987; Terry et al., *Immunology* 64:331, 1988).

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Enhancement of the Kinase Activity of p56^{lck} following anti-CD26 (1F7) Treatment

Recent studies have demonstrated that the cytoplasmic domain of CD45 has PTPase activity which
5 regulates T cell activation pathways through dephosphorylation of phosphotyrosine (Charboneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182, 1988; Ledbetter et al., *Proc. Natl. Acad. Sci., USA* 85:8628; Pingel et al., *Cell* 58:1055, 1989; Koretzky et al., *Nature* 346:66,
10 1990). One of the potential substrates for the CD45 PTPase is the tyrosine kinase p56^{lck} (Osergaard et al., *Proc. Natl. Acad. Sci. USA* 86:8959, 1989; Mustelin et al., *Proc. Natl. Acad. Sci. USA* 86:6302, 1989), which itself may be involved in the CD3 chain phosphorylation
15 (Veillette et al., *Nature* 338:257, 1989). CD26 may function in this system by enhancing CD3 phosphorylation through its association with CD45. If this model is correct, incubation with anti-CD26 (1F7) should alter p56^{lck} kinase activity as measured by *in vitro*
20 autophosphorylation.

To analyze *in vitro* kinase activity, samples of 3.0×10^7 T lymphocytes were incubated in culture media with anti-CD26 (1F7) for various periods of time at 37°C. Immunoprecipitation and kinase analysis was then carried
25 out as described by Rudal et al. (*Proc. Natl. Acad. Sci. USA* 85:5190, 1988). Cells were then solubilized in lysis buffer (1% NP-40, 20 mM TRIS-HCl [pH 8.0], 150 mM NaCl, 0.4 mM sodium vanadate, 0.5 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate, 1 mM PMSF) for 30 min at 4°C. CD4
30 was immunoprecipitated from lysates containing equivalent amounts of total protein (500 µg) by a combination of anti-CD4 (19thy5D7; IgG2) and protein A-Sepharose. The immunoprecipitates were then washed extensively with lysis buffer prior to incubation with 30 µl of 25 mM
35 Hepes containing 0.1% NP-40, and 10 µCi of [λ -³²P]ATP (ICN,

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Costa Mesa, CA). After incubation of 15-30 min at 25°C, the reaction was stopped by the addition of sample buffer and the reaction products were resolved on 9% SDS-PAGE.

As shown in Fig. 11, the PTK activity of p56^{lck} precipitated with CD4 significantly increased after 1 hour of incubation with anti-CD26 (lane 2) compared to a no-anti-CD26 control (lane 1). The kinase activity was even higher after 2, 3 or 4 hours of incubation with anti-CD26 (lanes 3-6, respectively). Concomitantly, the expression of CD26 on T cells treated with anti-CD26 (1F7) began to decrease within 1 hour of incubation and continued to decline as previously described (Dang et al., *J. Immunol.* 145:3936, 1990). Similar results were obtained when another anti-CD26 (Ta1) antibody was used. Nevertheless, incubation of cells with control anti-Class I MHC or anti-VLA 4 mAbs did not alter p56^{lck} activity. The above results support the notion that the interaction of CD26 with CD45 enhances p56^{lck} activity.

The kinetics of p56^{lck} PTK activity (Fig. 11) and tyrosine phosphorylation of CD3 (Fig. 6) showed a similar pattern. This similarity supports the conclusion that tyrosine phosphorylation of CD3 induced by anti-CD26 is related to the PTK activity of p56^{lck}. In addition, the similar kinetics also showed that the increase in p56^{lck} PTK activity quickly affects the phosphorylation of CD3, as reported previously (Veillett et al., *supra*). While the peak of the p56^{lck} PTK activity or phosphorylation of CD3 induced by various stimuli is observed within minutes (Vivier et al., *supra*; Veillette et al., *supra*), the peak of either p56^{lck} or CD3 phosphorylation induced by anti-CD26 treatment was observed after hours. In this regard, although the close relationship between CD45 PTPase activity and p56^{lck} PTK activity has been reported (Ostergaard et al., *supra*; Mustelin et al., *supra*; Veillette et al., *supra*), the regulation of PTPase

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activity of CD45 has not yet been established. Therefore, it is possible that the change in PTPase activity or the interaction between CD45 PTPase and p56^{lck} may require a relatively long time period following anti-
5 CD26 treatment. It is also possible that the interaction between CD45 PTPase and p56^{lck} is via an indirect rather than a direct mechanism.

CD26 is broadly distributed on non-hematopoietic cells. However, since the expression of CD45 is largely
10 restricted to leukocytes, the association between CD26 and CD45 is probably found only on leukocytes. On the other hand, membrane-linked PTPases such as CD45 have been found on non-hematopoietic cells (Streuli et al., *J. Exp. Med.* 168:1553, 1988; Streuli et al., *Proc. Natl.*
15 *Acad. Sci. USA* 86:8698, 1989; Lau et al. *Biochem J.* 257:23, 1989). Although the functional role of CD26 on nonhematopoietic cells is unclear, it is possible that CD26 is associated with the membrane-linked PTPase on nonhematopoietic cells.

20 In summary, we have demonstrated that anti-CD26-induced modulation resulted in enhanced CD3 phosphorylation and increased p56^{lck} PTK activity. Both observations are consistent with the enhanced proliferative response of T cells following CD26
25 modulation. These observations further suggest that the physical association of CD26 with CD45 may be key for CD26-mediated T cell signaling pathways. CD26 is known to be the membrane-associated ectoenzyme DPPIV which can cleave N-terminal dipeptides from polypeptides with
30 either L-proline or L-alanine at the penultimate position. Although the natural ligand for CD26/DPPIV has not yet been established, binding of the natural substrate to the DPPIV enzyme may lead to cleavage and alteration in the biologic activity of the ligand. In
35 light of the close proximity of the CD26 and CD45

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molecules, it is possible that CD26 modulates the enzymatic activity of the CD45 PTPase or perhaps affects the accessibility of critical substrates. This process would then enhance T cell activation via the CD3 or CD2
5 pathway and could amplify the immune response *in vivo*. It should also be noted that increased numbers of CD26+ T lymphocytes have been found in both inflamed tissues and peripheral blood of patients with multiple sclerosis, Graves' Disease and rheumatoid arthritis (Hafler et al.,
10 *N. Engl. J. Med.* 312:1405, 1985; Nakao et al., *J. Rheumatol.* 16:904, 1989; Eguchi et al., *J. Immunol.* 142:4233, 1989), suggesting that these CD26+ T cells may play an important role in chronic inflammation and in subsequent tissue damage.

15 Soluble CD26 Fragments

Soluble fragments of CD26 are useful for interfering with CD26 activity. The fact that CD26 is a type II membrane protein suggests certain strategies for designing soluble fragments. For type II membrane
20 proteins, the signal sequence used to transfer the protein across a membrane also serves as an anchor to the membrane. The cleavage of the signal sequence after protein transfer which usually occurs for other secreted proteins does not occur in type II transmembrane
25 proteins. Thus, soluble forms of CD26 can be prepared by making its signal/anchor sequence accessible to a cellular proteolytic cleavage system. To accomplish this, the putative signal sequence of CD26 was shortened, as described below, since the 23 amino acid CD26 signal
30 sequence is longer than most natural occurring cleavable signal sequences (von Heijne et al., *J. Mol. Biol.* 184:99, 1985). This is expected to result in proteolytic cleavage of the expressed polypeptide at or near one of the residues Ala Thr Ala corresponding to positions 35-37
35 of wild type CD26, yielding a soluble fragment of CD26

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having at its amino terminus Ala₃₅, Thr₃₆, Ala₃₇ or Asp₃₈ of wild type CD26.

A first soluble CD26 construct is created by deleting the codons corresponding to amino acids 3-9 of intact CD26 (shown as the boxed amino acids in Fig. 13). The amino terminal sequence of the expressed polypeptide is MKGLLG-- (SEQ ID NO: 4) rather than the original MKTPWKVLLGLLG-- (SEQ ID NO: 5), and the potential proteolytic cleavage sites are shown as arrows in Fig. 13. This deletion mutant is prepared by oligonucleotide directed mutagenesis (see below) using the following oligonucleotide:

5'-ACGCCGACGATGAAGGGACTGCTGGGTGCT-3' (SEQ ID NO: 6).

A second construct is generated by taking advantage of the following rules proposed for signal peptide cleavage: (1) the residue in position -1 must be small, i.e., either Ala, Ser, Gly, Thr, Cys, Gln; (2) the residue in position -3 must not be aromatic (Phe, His, Tyr, Trp), charged (Asp, Glu, Lys, Arg), or large and polar (Asn, Gln); and (3) Pro must not be present at positions -3 through -1 (von Heijne, *Nuc. Acids Res.* 14:4683, 1986). Following these rules, we have designed a CD26 cDNA construct lacking codons corresponding to amino acids 24 to 34 of wild type CD26 (illustrated as the boxed amino acids in Fig. 14). This deletion mutant encodes the amino acid sequence

--IITVATADSR-- (SEQ ID NO: 7) instead of the original --IITVPVLLNKGTDDATADSR-- (SEQ ID NO: 8), and the potential proteolytic cleavage sites are shown as arrows in Fig. 14. This mutant is prepared by oligonucleotide-directed mutagenesis (see below) using the following oligonucleotide: 5'-ACCATCATCACCGTGGCTACAGCTGACAGT-3' (SEQ ID NO: 9).

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Site-directed mutagenesis is performed as follows. The 3.0 kb CD26 cDNA fragment generated by the *Xba*I treatment of the original plasmid CDM7-CD26 is inserted into the *Xba*I site of pTZ19u (Bio-rad). A recombinant
5 plasmid which inserts the cDNA inverse to the *lacZ* gene on the plasmid is identified by restriction enzyme mapping and used for subsequent mutagenesis.

Using single-stranded DNA prepared from this plasmid as a template and the previously-described
10 oligonucleotides as primers, oligonucleotide-directed mutagenesis is performed by the method of Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985), using a commercially available kit (BioRad, Richmond, CA).

To obtain high level expression of soluble CD26, a
15 new expression vector is constructed. First the *Xba*I CD26 cDNA fragment of pTZ19u-CD26 and the *Hind*III-*Xba*I vector fragment of Rc/CMV (Invitrogene, San Diego, CA) are treated with Klenow enzyme and ligated. The resulting plasmid is screened by restriction enzyme
20 mapping for the insertion of the CD26 cDNA fragment under the control of the CMV promoter. This construct leaves one *Xba*I site just in front of the CD26 cDNA. Then, the *Mlu*I-*Xba*I CMV promoter DNA fragment of this plasmid DNA is exchanged with the *Hind*III-*Xba*I SR α promoter DNA
25 fragment of pSR α -26 to give a final expression vector RcSR α -26. Next, the above mutant CD26 cDNAs are transferred to this expression vector. The *Xba*I-*Dra*III DNA fragment derived from the mutant cDNAs which encoded the mutant part and the wild type 2.0 kb *Dra*III-*Hind*III
30 DNA fragment are ligated with the *Xba*I-*Hind*III vector fragment of RcSR α -26. The expression plasmid which has the Δ 3-9 or Δ 24-34 mutant CD26 cDNA is identified by restriction enzyme mapping and DNA sequencing. The resultant plasmids RcSR α -26. Δ 3-9 and RcSR α -26. Δ 24-34 are
35 used to transfect Jurkat cells or CHO cells.

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- Jurkat cells are transfected with these plasmids as described above except pSVneo-sp is omitted from the donor DNA mixture since the RcSR α plasmid already carries the neo resistance marker. Neo-resistant clones are
- 5 screened by metabolic labelling and immunoprecipitation (Harlow et al., eds. *Antibodies: a laboratory manual*, Cold Spring Harbor Laboratory, 1988) for the expression of soluble CD26. The transfectants which produce a large amount of soluble CD26 are used for protein production.
- 10 CHO cells transfected with the DNA mixture of pMT2 and RcSR α -26. Δ 3-9 or RcSR α -26. Δ 24-34 are selected for their growing ability in α -medium and the production of soluble CD26. The expression of the soluble protein is amplified by culturing the transfected CHO cells in
- 15 medium containing an increasing amount of MTX. Although both Jurkat cells and CHO cells can provide the soluble form of CD26, the protein produced by Jurkat cells is preferred because of its human T cell origin.

Another approach to making fragments of CD26 is
20 illustrated by the following:

Ligation of the CD26 XbaI-SphI cDNA fragment to the vector RcSR α -26 XbaI-HindIII DNA fragment and the following synthetic DNA linker:

5'-----CATAGTAATCGATA

- 25 GTACGTATCATTAGCTATTCGA-----5' (SEQ ID NO: 10) introduces an in-frame stop codon that results in deletion of the segment of CD26 from amino acid 594 to the carboxy terminus of the wild-type protein. This deletion mutant, which is shown in Fig. 15 (SEQ ID NO:
- 30 11), lacks the putative catalytic site of CD26 and has a new carboxy terminus of --GDKIMHA (SEQ ID NO: 12).

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CD26 Derivatives Capable of Disrupting CD26/CD45
Interaction

Other polypeptide fragments of CD26 can be produced by standard methods of protein synthetic chemistry, using the information disclosed herein to design appropriate polypeptides and assay them for biological activity. A preferred method of producing such fragments, however, is by the use of recombinant DNA techniques. For example, the sequence of CD26 given in Fig. 1 (SEQ ID NO:1) can be used to design oligonucleotides encoding fragments of CD26 containing deletions of nonessential CD26 amino acid residues from the beginning, the end, and/or any central portion of the protein; such oligonucleotides are chemically synthesized by known methods and inserted into expression vectors for expression of a polypeptide fragment of CD26. Alternatively, one may manipulate the CD26 coding regions of CD26 expression plasmids by site-directed mutagenesis, as disclosed above for two such fragments of CD26, or by insertion of a stop codon at an appropriate place in the coding sequence. The CD26 fragment can then be produced in transfected cultured cells in large quantities, purified by standard methods, and tested in an assay such as the immunoprecipitation assay described above, which is useful for identifying fragments capable of disrupting the interaction of CD26 and CD45. Briefly, surface-labeled peripheral blood T cells which express both CD26 and CD45 (or any mammalian cells transfected with cDNAs encoding CD26 and CD45 so that both proteins are functionally expressed on the cells' surfaces) are incubated in the presence and absence of a CD26 polypeptide fragment. The cells are lysed in digitonin lysis buffer, and anti-CD45 monoclonal antibody is used to immunoprecipitate CD45 and any proteins associated

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with CD45. The amount of CD26 that co-precipitates with CD45 in the presence of a given polypeptide fragment can be determined by known methods (e.g., by densitometer readings of the labelled bands on an SDS-PAGE gel

5 analyzing the constituents of an immunoprecipitate) and compared to the amount that co-precipitates with CD45 in the absence of the polypeptide fragment. Alternatively, one can instead use an anti-CD26 antibody and measure the relative amounts of CD45 that co-precipitate with CD26 in

10 the presence and absence of the given polypeptide fragment. If an anti-CD26 antibody is used, it is preferred that the antibody does not substantially bind to the competitor CD26 polypeptide; such binding interferes with the assay. In either case, CD26

15 polypeptide fragments which interfere with the interaction between CD26 and CD45 will decrease co-precipitation.

An analysis similar to that described above can be used to identify polypeptide fragments of CD45 which

20 disrupt CD26/CD45 interaction. When screening CD45 fragments, it is preferable to perform the immunoprecipitation with anti-CD26 antibody.

Association of p43 with CD26

When CD26 is immunoprecipitated from surface-

25 labelled T cells and the immunoprecipitate is analyzed on SDS-PAGE, two bands are typically seen: one at 110kDa, corresponding to CD26, and a second, much fainter band at 43kDa. This lower molecular weight protein is termed "p43". Fig. 12 illustrates one such experiment, in which

30 E+ cells were labeled by lactoperoxidase-catalyzed iodination and lysed in NP-40 lysis buffer for immunoprecipitation as described above. Precipitates were analyzed by 9% SDS-PAGE. Lane 1: anti-CD1 (T6) as negative control; lane 2: anti-1F7; lane 3: anti-Ta1;

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lane 4: anti-5F8 (another anti-CD26 monoclonal antibody);
lane 5: anti-CD29 (4B4) as control. As shown in Fig. 12,
anti-1F7 brought down an obvious 43kDa structure (lane 2)
from surface-labeled T cells. On the other hand, this
5 structure was detected faintly following anti-Ta1 or
anti-5F8 precipitation (lanes 3 and 4). This band was
not detected following anti-CD1 or anti-CD29
precipitation (lanes 1 and 5). Similar results were seen
when the cells were human thymocytes or from the human T
10 cell lines H9 or Peer IV (data not shown). In other
anti-Ta1 or anti-5F8 immunoprecipitation experiments
using T cells from other donors, the 43kDa band was
sometimes more distinct than those shown in lanes 3 and 4
of Fig. 12. In addition, a third band at approximately
15 70 kDa is sometimes observed in these CD26
immunoprecipitation experiments. Because they are found
in association with the 110 kDa CD26 molecule, both the
43 kDa molecule and the 70 kDa molecule may play
important roles in T cell activation. Compounds (such as
20 fragments of CD26) which interfere with the association
of CD26 with either p43 or the 70 kDa molecule may be
detected by means of a screening assay patterned on those
described above with respect to CD26 and CD45.

It is thought to be unlikely that anti-1F7 cross-
25 reacts with p43, since the density of the 43kDa band
decreased after repeated preclearing by either anti-Ta1
or anti-5F8. Although the reasons for the variability in
the detection of p43 are not clear, it is possible that
the binding of anti-CD26 mAbs may generate conformational
30 changes in CD26, affecting the association of the 43 kDa
molecule with the 110 kDa molecule. It is also possible
that the Ta1 or 5F8 epitope may be close to the
association site between the 43 and 110 kDa molecules,
such that binding of these mAbs may inhibit the
35 association of these molecules with each other.

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P43 may be purified by affinity chromatography, using an anti-CD26 monoclonal antibody to purify the CD26-p43 complex from T cell membranes. P43 may then be separated from CD26 by SDS-PAGE, followed by HPLC if
5 further purification is necessary. Affinity chromatography with monoclonal antibodies, SDS-PAGE, and HPLC are all standard methods well known to those of ordinary skill in the art.

Hybridization probes based upon a partial amino
10 acid sequence of the purified protein may be used to select p43 cDNA from a T cell library. Alternatively, the partial amino acid sequence can be used to design PCR primers for priming synthesis of a partial p43 cDNA on mRNA templates, using standard methods, and the resulting
15 partial cDNA used as a probe to detect full-length p43 cDNA in a T cell library. This cDNA can be inserted in an expression plasmid and used to transfect cells which do not naturally express the p43 gene. Such cells may be used as an antigen to develop anti-p43 monoclonal
20 antibodies, and also as a means to study the role of p43 in T cell activation. They can also be used in the screening assay referred to above.

Northern Analysis Using a CD26 cDNA Probe

Analysis of the degree of expression of CD26 in
25 any given cell type or tissue type can be accomplished using the standard technique of Northern blotting, probing with a labelled, single stranded nucleic acid molecule derived from the coding region of CD26 cDNA. The probe would have a sequence based upon the sense
30 strand of SEQ ID NO: 1, which is complementary to CD26 mRNA, and preferably would be at least 8 nucleotides in length (more preferably at least 14 nucleotides, and most preferably at least 30). The probe may contain most or all of the entire coding sequence of CD26 cDNA. Such an

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assay, which would be useful for diagnosing conditions characterized by the over- or under-expression of CD26 in a given cell type, such as T cells, would include the following steps:

- 5 (a) providing a biological sample containing mRNA of a cell;
 - (b) contacting the sample with a single-stranded nucleic acid probe as described above; and
 - (c) detecting hybridization of the probe with the
- 10 sample, which hybridization would be indicative of the presence of CD26 mRNA in the cell.

Purification of Soluble CD26

- To produce soluble CD26, CHO cells stably expressing CD26Δ3-9 (CD26 deleted for amino acids 3-9)
- 15 were cultured in serum-free medium (CHO-S-SFM; GIBCO/BRL) containing 0.5 μM methotrexate. The culture supernatant was collected and proteins were precipitated using 75% ammonium sulfate. The resulting pellet was solubilized in PBS, dialyzed against PBS, and loaded on a ConA-
- 20 Sepharose column (Pharmacia, Piscataway, NJ) equilibrated with 2xPBS/0.02% sodium azide. The column was washed with the equilibration buffer, and protein was eluted with 2xPBS/0.2M methyl α-D-mannopyranoside/0.02% sodium azide. A DPPIV assay (described below) was used to
- 25 identify the CD26-containing fractions, which were pooled and loaded directly on a BSA-conjugated Affigel™ 10 column (Bio-rad) equilibrated with PBS/0.02% sodium azide. The flowthrough fraction was collected and applied to a 1F7-conjugated Affigel™ 10 column
- 30 equilibrated with PBS. The column was washed with PBS, and soluble CD26 was eluted with PBS/3M sodium thiocyanate. The fractions containing DPPIV activity were pooled and dialyzed against PBS. The resulting

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soluble CD26 was more than 95% pure as judged by gel electrophoresis.

Soluble CD26 and Cell Activation

Soluble CD26 was shown to stimulate antigen-
5 dependent proliferation of peripheral blood lymphocytes
in vitro. Assays were performed in triplicate wells in
round-bottom plates using 0.2 ml/well standard culture
medium consisting of RPMI1640 supplemented with 10% human
AB serum, 4 mM L-glutamine, 25 mM HEPES buffer
10 (Microbiological Associates), 0.5% sodium bicarbonate,
and 50 µg/ml of Gentamicin (GIBCO). The cell
concentration was 1.2×10^5 cells/well. Tetanus toxoid
(Connaught Lab, Inc.) dialyzed against PBS was added to
some of the wells to make a final concentration of 0.2 or
15 0.1 L.T. unit/ml (1/40 or 1/80 dilution of the original
solution, respectively); the toxoid serves as soluble
antigen in this assay. Purified soluble CD26 antigen,
soluble LCA (leucocyte common antigen; CD45), or soluble
CD4 was added at a final concentration of 1 µg/ml or 25
20 µg/ml. After 7 days culture in a CO₂ incubator, the
cells were pulsed with 1 µCi/well of ³H-thymidine. After
a 16 hr incubation, the cells were harvested and the ³H-
thymidine incorporation was measured using a
scintillation counter.

25 As shown in Fig. 16, in the presence of tetanus
toxoid (diluted 80-fold or 40-fold), soluble CD26
(prepared as described above) stimulated PBL
proliferation in a dose-dependent manner. This
stimulation was greater than that observed when soluble
30 CD45 or soluble CD4 was used instead of soluble CD26.

This assay can be used to screen fragments of CD26
to identify molecules capable of stimulating antigen-
dependent immune cell proliferation. In addition, it can
be used to assay for compounds capable of inhibiting
35 soluble CD26-stimulated proliferation of lymphocytes. By

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substituting an anti-CD3 antibody such as OKT3 (Kung et al. U.S. Patent 4,658,019, 4,361,549, and 4,654,210) for the tetanus toxoid in this assay, the ability of fragments of CD26 to stimulate antigen-independent immune cell proliferation can be determined.

CD26 Mutant

Standard methods of site-directed mutagenesis were used to produce a point mutation (Ser⁶²⁹→Ala) within the putative catalytic site of DPPIV activity of CD26. The enzymatic activity of the resulting mutant CD26 (CD26-629A) was examined by transfecting Jurkat cells with plasmids expressing intact CD26, CD26-629A, or vector only. Transformed cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, 50 µg/ml Gentamicin, and 0.25 mg/ml Geneticin (GIBCO), then harvested, lysed, and separated into a membrane fraction and a cytosol fraction (by the method of Siekierka et al., J. Immunol. 143:1580-1583, 1989). DPPIV activity of each fraction was measured in accordance with Hanski et al. (Exp. Cell Res. 178:64-72, 1988). Membranes from cells transformed with the plasmid encoding CD26-629A had almost no DPPIV activity, while membranes containing wild-type CD26 had substantial DPPIV activity. In addition, some DPPIV activity was observed in the cytosolic fraction of cells expressing wild-type CD26, but not cells expressing CD26-629A.

Although CD26-629A apparently lacks DPPIV activity, cells expressing CD26-629A were recognized by three anti-CD26 antibodies (1F7, Ta1(4EL), and 5F8) which recognize wild-type CD26, indicating that the mutant protein is expressed in the transformed cells, and suggesting that the mutation does not have a substantial effect on protein conformation.

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Jurkat cells expressing wild-type CD26 were stimulated with anti-CD3 antibody (OKT3) and either anti-CD26 antibody (1F7) or PMA produced substantially more IL-2 than cells expressing CD26-629A or cells transformed with vector only. This suggests that the DPPIV activity of CD26 is important for both CD26-dependent and CD26-independent activation. It was also observed that, after stimulation with anti-CD26 and anti-CD3 antibodies, cells expressing the mutant form of CD26 produced more IL-2 than control cells that did not express either mutant or wild-type CD26, suggesting that DPPIV activity is not the only facet of CD26 which contributes to stimulation of IL-2 production in this system.

Use

Soluble CD26 and variants thereof are generally useful as immune response-stimulating therapeutics. For example, the compounds of the invention can be used for treatment of disease conditions characterized by immunosuppression: e.g., AIDS or AIDS-related complex, other virally- or environmentally-induced conditions, and certain congenital immune deficiencies. The compounds may also be employed to increase immune function that has been impaired by the use of immunosuppressive drugs such as certain chemotherapeutic agents, and therefore are particularly useful when given in conjunction with such drugs. When given as an adjuvant in conjunction with a vaccine antigen, the compounds of the invention will boost the immune response triggered by the vaccine and thus increase the vaccine's protective potency. This would be particularly beneficial where the vaccinee is incapable of generating an optimal immune response without the use of such an adjuvant, as is the case for newborns or for persons undergoing renal dialysis or transplantation, or where the vaccine antigen is one which is poorly immunogenic.

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- Generally, the compounds of the invention will be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, or intraperitoneally. Optimal formulation and dosage can be readily determined by one of ordinary skill in the art of pharmacology, taking into account such factors as the biological half-life of the compound and the degree of immunostimulation desired.
- 10 It is expected that a typical dose for a severely immunocompromised patient will be approximately 0.01 to 100 $\mu\text{g/kg/day}$. When utilized as a vaccine adjuvant, a typical single dose of the compound of the invention would be 0.1 to 100 μg .
- 15 Instead of soluble forms of CD26, intact CD26 or a form of CD26 which retains the membrane-anchoring amino terminal portion of native CD26, as well as all critical portions of the remainder of the molecule, can be incorporated into red cell "ghosts" or liposomes, so that
- 20 the protein is expressed on the surface of the ghosts or liposomes. This form of CD26 is then suspended in a pharmaceutically acceptable carrier and introduced into the patient as described above, so that it can interact with the patient's immunological cells *in vivo*.
- 25 Alternatively, peripheral blood lymphocytes can be withdrawn from the patient and treated with a CD26 compound of the invention (whether in soluble or membrane-bound form, or attached to a solid support by standard methodologies) *ex vivo*, prior to introducing the
- 30 newly-stimulated lymphocytes into the same or a different patient.

As discussed above, the assay for enhancement of lymphocyte proliferation with soluble CD26 can be utilized to screen for compounds which inhibit such

35 enhancement, and which therefore could be used to

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interfere with CD26-stimulated proliferation of lymphocytes *in vivo*. The information provided above concerning the location of the DPPIV active site of CD26 provides a starting place for the design of compounds which will bind to the active site and thus potentially inhibit the stimulatory activity of CD26. Such compounds can first be tested for their ability to bind to CD26 by passing each such compound over a CD26 affinity column; compounds which bind to the column can then be assayed for their ability to inhibit soluble CD26-enhanced proliferation of lymphocytes *in vitro*, as described above. Such inhibitory compounds would be useful for the treatment of conditions characterized by an unwanted immune response: for example, autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis.

Other Embodiments

The invention also includes analogs of CD26 and of fragments of CD26. The term "analogs" refers to polypeptide fragments of CD26 having conservative and/or non-conservative substitutions for some of the amino acids of naturally-occurring CD26, having D-amino acids in place of some or all of the corresponding L-amino acids, or having non-peptide bonds in place of some of the peptide bonds of CD26. Techniques for producing such analogs are well known in the art, and can be readily accomplished by those of ordinary skill. Preferably at least 85%, more preferably at least 95%, and most preferably at least 99%, of the amino acids in the analog are identical to the corresponding ones in CD26. It is important that the substitutions do not eliminate the ability of the polypeptide fragment to interfere with the naturally occurring association between CD26 and CD45, or the ability of the compound to stimulate proliferation of lymphocytes. In some instances, the removal of peptide bonds from a polypeptide compound is a desirable goal.

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because the presence of such bonds may leave the compound susceptible to attack by proteolytic enzymes.

Additionally, such peptide bonds may affect the biological availability of the resulting therapeutic
5 molecules. The removal of peptide bonds is part of a process referred to as "depeptidization".

Depeptidization entails such modifications as replacement of the peptide bond (-CONH-) between two given amino acids with a spatially similar group such as -CH₂CH₂-, -
10 CH₂-O-, -CH=CH-or -CH₂S-, generally by incorporating a non-peptide mimetic of the dipeptide into the chemically synthesized analog of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Tanaka, Toshiaki

(ii) TITLE OF INVENTION: HUMAN CD26 AND METHODS FOR USE

(iii) NUMBER OF SEQUENCES: 16

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(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2924
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACGCCGACG	ATG	AAG	ACA	CCG	TGG	AAG	GTT	CTT	CTG	GGA	CTG	CTG	GGT	49
	Met	Lys	Thr	Pro	Trp	Lys	Val	Leu	Leu	Gly	Leu	Leu	Gly	
	1				5					10				
GCT GCT GCG CTT GTC ACC ATC ATC ACC GTG CCC GTG GTT CTG CTG AAC	97													
Ala Ala Ala Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn														
15 20 25														
AAA GGC ACA GAT GAT GCT ACA GCT GAC AGT CGC AAA ACT TAC ACT CTA	145													
Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu														
30 35 40 45														
ACT GAT TAC TTA AAA AAT ACT TAT AGA CTG AAG TTA TAC TCC TTA AGA	193													
Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg														
50 55 60														
TGG ATT TCA GAT CAT GAA TAT CTC TAC AAA CAA GAA AAT AAT ATC TTG	241													
Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu														
65 70 75														
GTA TTC AAT GCT GAA TAT GGA AAC AGC TCA GTT TTC TTG GAG AAC AGT	289													
Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser														
80 85 90														
ACA TTT GAT GAG TTT GGA CAT TCT ATC AAT GAT TAT TCA ATA TCT CCT	337													
Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro														
95 100 105														
GAT GGG CAG TTT ATT CTC TTA GAA TAC AAC TAC GTG AAG CAA TGG AGG	385													
Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg														
110 115 120 125														
CAT TCC TAC ACA GCT TCA TAT GAC ATT TAT GAT TTA AAT AAA AGG CAG	433													
His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln														
130 135 140														
CTG ATT ACA GAA GAG AGG ATT CCA AAC AAC ACA CAG TGG GTC ACA TGG	481													
Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp														
145 150 155														
TCA CCA GTG GGT CAT AAA TTG GCA TAT GTT TGG AAC AAT GAC ATT TAT	529													
Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr														
160 165 170														
GTT AAA ATT GAA CCA AAT TTA CCA AGT TAC AGA ATC ACA TGG ACG GGG	577													
Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly														
175 180 185														
AAA GAA GAT ATA ATA TAT AAT GGA ATA ACT GAC TGG GTT TAT GAA GAG	625													
Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu														
190 195 200 205														

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GAA GTC TTC AGT GCC TAC TCT GCT CTG TGG TGG TCT CCA AAC GGC ACT	673
Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr	
210 215 220	
TTT TTA GCA TAT GCC CAA TTT AAC GAC ACA GAA GTC CCA CTT ATT GAA	721
Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu	
225 230 235	
TAC TCC TTC TAC TCT GAT GAG TCA CTG CAG TAC CCA AAG ACT GTA CGG	769
Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg	
240 245 250	
GTT CCA TAT CCA AAG GCA GGA GCT GTG AAT CCA ACT GTA AAG TTC TTT	817
Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Val Lys Phe Phe	
255 260 265	
GTT GTA AAT ACA GAC TCT CTC AGC TCA GTC ACC AAT GCA ACT TCC ATA	865
Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile	
270 275 280 285	
CAA ATC ACT GCT CCT GCT TCT ATG TTG ATA GGG GAT CAC TAC TTG TGT	913
Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys	
290 295 300	
GAT GTG ACA TGG GCA ACA CAA GAA AGA ATT TCT TTG CAG TGG CTC AGG	961
Asp Val Thr Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg	
305 310 315	
AGG ATT CAG AAC TAT TCG GTC ATG GAT ATT TGT GAC TAT GAT GAA TCC	1009
Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser	
320 325 330	
AGT GGA AGA TGG AAC TGC TTA GTG GCA CGG CAA CAC ATT GAA ATG AGT	1057
Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser	
335 340 345	
ACT ACT GGC TGG GTT GGA AGA TTT AGG CCT TCA GAA CCT CAT TTT ACC	1105
Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr	
350 355 360 365	
CTT GAT GGT AAT AGC TTC TAC AAG ATC ATC AGC AAT GAA GAA GGT TAC	1153
Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr	
370 375 380	
AGA CAC ATT TGC TAT TTC CAA ATA GAT AAA AAA GAC TGC ACA TTT ATT	1201
Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile	
385 390 395	
ACA AAA GGC ACC TGG GAA GTC ATC GGG ATA GAA GCT CTA ACC AGT GAT	1249
Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp	
400 405 410	
TAT CTA TAC TAC ATT AGT AAT GAA TAT AAA GGA ATG CCA GGA GGA AGG	1297
Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg	
415 420 425	
AAT CTT TAT AAA ATC CAA CTT AGT GAC TAT ACA AAA GTG ACA TGC CTC	1345
Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu	
430 435 440 445	

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AGT TGT GAG CTG AAT CCG GAA AGG TGT CAG TAC TAT TCT GTG TCA TTC	1393
Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe	
450 455 460	
AGT AAA GAG GCG AAG TAT TAT CAG CTG AGA TGT TCC GGT CCT GGT CTG	1441
Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu	
465 470 475	
CCC CTC TAT ACT CTA CAC AGC AGC GTG AAT GAT AAA GGG CTG AGA GTC	1489
Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val	
480 485 490	
CTG GAA GAC AAT TCA GCT TTG GAT AAA ATG CTG CAG AAT GTC CAG ATG	1537
Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met	
495 500 505	
CCC TCC AAA AAA CTG GAC TTC ATT ATT TTG AAT GAA ACA AAA TTT TGG	1585
Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp	
510 515 520 525	
TAT CAG ATG ATC TTG CCT CCT CAT TTT GAT AAA TCC AAG AAA TAT CCT	1633
Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro	
530 535 540	
CTA CTA TTA GAT GTG TAT GCA GGC CCA TGT AGT CAA AAA GCA GAC ACT	1681
Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr	
545 550 555	
GTC TTC AGA CTG AAC TGG GCC ACT TAC CTT GCA AGC ACA GAA AAC ATT	1729
Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile	
560 565 570	
ATA GTA GCT AGC TTT GAT GGC AGA GGA AGT GGT TAC CAA GGA GAT AAG	1777
Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys	
575 580 585	
ATC ATG CAT GCA ATC AAC AGA AGA CTG GGA ACA TTT GAA GTT GAA GAT	1825
Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp	
590 595 600 605	
CAA ATT GAA GCA GCC AGA CAA TTT TCA AAA ATG GGA TTT GTG GAC AAC	1873
Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn	
610 615 620	
AAA CGA ATT GCA ATT TGG GGC TGG TCA TAT GGA GGG TAC GTA ACC TCA	1921
Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser	
625 630 635	
ATG GTC CTG GGA TCA CCA AGT GGC GTG TTC AAG TGT GGA ATA GCC GTG	1969
Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val	
640 645 650	
GCG CCT GTA TCC CGG TGG GAG TAC TAT GAC TCA GTG TAC ACA GAA CGT	2017
Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg	
655 660 665	
TAC ATG GGT CTC CCA ACT CCA GAA GAC AAC CTT GAC CAT TAC AGA AAT	2065
Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn	
670 675 680 685	
TCA ACA GTC ATG AGC AGA GCT GAA AAT TTT AAA CAA GTT GAG TAC CTC	2113
Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu	
690 695 700	

- 49 -

CTT ATT CAT GGA ACA GCA GAT GAT AAC GTT CAC TTT CAG CAG TCA GCT 2161
 Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala
 705 710 715

CAG ATC TCC AAA GCC CTG GTC GAT GTT GGA GTG GAT TTC CAG GCA ATG 2209
 Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met
 720 725 730

TGG TAT ACT GAT GAA GAC CAT GGA ATA GCT AGC AGC ACA GCA CAC CAA 2257
 Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln
 735 740 745

CAT ATA TAT ACC CAC ATG AGC CAC TTC ATA AAA CAA TGT TTC TCT TTA 2305
 His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu
 750 755 760 765

CCT TAGCACCTCA AAATACCATG CCATTAAAG CTTATTAAAA CTCATTTTGT 2358
 Pro

TTTTCATTAT CTCAAAACCTG CACTGTCAAG ATGATGATGA TCTTTAAAT ACACACTCAA 2418
 ATCAAGAAAC TTAAGGTAC CTTTGTCCCA AAATTTTATA CCTATCATCT TAAGTAGGGA 2478
 CTTCTGTCTT CACAACAGAT TATTACCTTA CAGAAGTTTG AATTATCCGG TCGGGTTTAA 2538
 TTGTTTAAAA TCATTTCTGC ATCAGCTGCT GAAACAACAA ATAGGAATTG TTTTATGGA 2598
 GGCTTTGCAT AGATCCCTG AGCAGGATTT TAATCTTTT CTAAGTGGAC TGGTTCAAAT 2658
 GTTGTCTCT TCTTTAAAGG GATGGCAAGA TGTGGGCAGT GATGTCATA GGGCAGGGAC 2718
 AGGATAAGAG GGATTAGGGA GAGAAGATAG CAGGGCATGG CTGGGAACCC AAGTCCAAGC 2778
 ATACCAACAC GACCAGGCTA CTGTCAGCTC CCCTCGGAGA AAAGTGTGCA GTCTGCGTGT 2838
 GAACAGCTCT TCTCCTTTAG AGCACAATGG ATCTCGAGGG ATCTTCCATA CCTACCAAGT 2898
 CTGCGCCTCG AGGCCGCGAC TCTAGA 2924

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 759
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Gly Leu Leu Gly Ala Ala Ala Leu Val Thr Ile Ile Thr Val
 1 5 10 15
 Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser
 20 25 30
 Arg Lys Thr Tyr Thr Leu Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu
 35 40 45
 Lys Leu Tyr Ser Leu Arg Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys
 50 55 60

- 50 -

Gln Glu Asn Asn Ile Leu Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser
 65 70 75 80
 Val Phe Leu Glu Asn Ser Thr Phe Asp Glu Phe Gly His Ser Ile Asn
 85 90 95
 Asp Tyr Ser Ile Ser Pro Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn
 100 105 110
 Tyr Val Lys Gln Trp Arg His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr
 115 120 125
 Asp Leu Asn Lys Arg Gln Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn
 130 135 140
 Thr Gln Trp Val Thr Trp Ser Pro Val Gly His Lys Leu Ala Tyr Val
 145 150 155 160
 Trp Asn Asn Asp Ile Tyr Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr
 165 170 175
 Arg Ile Thr Trp Thr Gly Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr
 180 185 190
 Asp Trp Val Tyr Glu Glu Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp
 195 200 205
 Trp Ser Pro Asn Gly Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr
 210 215 220
 Glu Val Pro Leu Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln
 225 230 235 240
 Tyr Pro Lys Thr Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val Asn
 245 250 255
 Pro Thr Val Lys Phe Phe Val Val Asn Thr Asp Ser Leu Ser Ser Val
 260 265 270
 Thr Asn Ala Thr Ser Ile Gln Ile Thr Ala Pro Ala Ser Met Leu Ile
 275 280 285
 Gly Asp His Tyr Leu Cys Asp Val Thr Trp Ala Thr Gln Glu Arg Ile
 290 295 300
 Ser Leu Gln Trp Leu Arg Arg Ile Gln Asn Tyr Ser Val Met Asp Ile
 305 310 315 320
 Cys Asp Tyr Asp Glu Ser Ser Gly Arg Trp Asn Cys Leu Val Ala Arg
 325 330 335
 Gln His Ile Glu Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg Pro
 340 345 350
 Ser Glu Pro His Phe Thr Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile
 355 360 365
 Ser Asn Glu Glu Gly Tyr Arg His Ile Cys Tyr Phe Gln Ile Asp Lys
 370 375 380
 Lys Asp Cys Thr Phe Ile Thr Lys Gly Thr Trp Glu Val Ile Gly Ile
 385 390 395 400

- 51 -

Glu Ala Leu Thr Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys
 405 410 415
 Gly Met Pro Gly Gly Arg Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr
 420 425 430
 Thr Lys Val Thr Cys Leu Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln
 435 440 445
 Tyr Tyr Ser Val Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg
 450 455 460
 Cys Ser Gly Pro Gly Leu Pro Leu Tyr Thr Leu His Ser Ser Val Asn
 465 470 475 480
 Asp Lys Gly Leu Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Lys Met
 485 490 495
 Leu Gln Asn Val Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu
 500 505 510
 Asn Glu Thr Lys Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe Asp
 515 520 525
 Lys Ser Lys Lys Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys
 530 535 540
 Ser Gln Lys Ala Asp Thr Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu
 545 550 555 560
 Ala Ser Thr Glu Asn Ile Ile Val Ala Ser Phe Asp Gly Arg Gly Ser
 565 570 575
 Gly Tyr Gln Gly Asp Lys Ile Met His Ala Ile Asn Arg Arg Leu Gly
 580 585 590
 Thr Phe Glu Val Glu Asp Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys
 595 600 605
 Met Gly Phe Val Asp Asn Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr
 610 615 620
 Gly Gly Tyr Val Thr Ser Met Val Leu Gly Ser Gly Ser Gly Val Phe
 625 630 635 640
 Lys Cys Gly Ile Ala Val Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp
 645 650 655
 Ser Val Tyr Thr Glu Arg Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn
 660 665 670
 Leu Asp His Tyr Arg Asn Ser Thr Val Met Ser Arg Ala Glu Asn Phe
 675 680 685
 Lys Gln Val Glu Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val
 690 695 700
 His Phe Gln Gln Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val Gly
 705 710 715 720
 Val Asp Phe Gln Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile Ala
 725 730 735

- 52 -

Ser Ser Thr Ala His Gln His Ile Tyr Thr His Met Ser His Phe Ile
 740 745 750

Lys Gln Cys Phe Ser Leu Pro
 755

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 755
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
 1 5 10 15
 Leu Val Thr Ile Ile Thr Val Ala Thr Ala Asp Ser Arg Lys Thr Tyr
 20 25 30
 Thr Leu Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser
 35 40 45
 Leu Arg Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn
 50 55 60
 Ile Leu Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu
 65 70 75 80
 Asn Ser Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile
 85 90 95
 Ser Pro Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln
 100 105 110
 Trp Arg His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys
 115 120 125
 Arg Gln Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val
 130 135 140
 Thr Trp Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp
 145 150 155 160
 Ile Tyr Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp
 165 170 175
 Thr Gly Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr
 180 185 190
 Glu Glu Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn
 195 200 205
 Gly Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu
 210 215 220
 Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr
 225 230 235 240

- 53 -

Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys
 245 250 255
 Phe Phe Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr
 260 265 270
 Ser Ile Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr
 275 280 285
 Leu Cys Asp Val Thr Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp
 290 295 300
 Leu Arg Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp
 305 310 315 320
 Glu Ser Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu
 325 330 335
 Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His
 340 345 350
 Phe Thr Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu
 355 360 365
 Gly Tyr Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr
 370 375 380
 Phe Ile Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr
 385 390 395 400
 Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly
 405 410 415
 Gly Arg Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr
 420 425 430
 Cys Leu Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val
 435 440 445
 Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro
 450 455 460
 Gly Leu Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu
 465 470 475 480
 Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val
 485 490 495
 Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys
 500 505 510
 Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys
 515 520 525
 Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala
 530 535 540
 Asp Thr Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu
 545 550 555 560
 Asn Ile Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly
 565 570 575

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Asp Lys Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val
 580 585 590
 Glu Asp Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val
 595 600 605
 Asp Asn Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val
 610 615 620
 Thr Ser Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile
 625 630 635 640
 Ala Val Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr
 645 650 655
 Glu Arg Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr
 660 665 670
 Arg Asn Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu
 675 680 685
 Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln
 690 695 700
 Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln
 705 710 715 720
 Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala
 725 730 735
 His Gln His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe
 740 745 750
 Ser Leu Pro
 755

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Lys Gly Leu Leu Gly
 1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGCCGACGA TGAAGGGACT GCTGGGTGCT 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Ile Thr Val Ala Thr Ala Asp Ser Arg
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala
1 5 10 15

Thr Ala Asp Ser Arg
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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ACCATCATCA CCGTGGCTAC AGCTGACAGT 30

(i) SEQUENCE CHARACTERISTICS:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTACGTATCA TTAGCTATTC GA 22

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 593
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
1 5 10 15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr
20 25 30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
35 40 45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
50 55 60

Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn
65 70 75 80

Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp
85 90 95

Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln
100 105 110

Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr
115 120 125

Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr
130 135 140

Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val
145 150 155 160

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Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile
 165 170 175
 Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp
 180 185 190
 Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe
 195 200 205
 Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala
 210 215 220
 Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe
 225 230 235 240
 Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr
 245 250 255
 Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn
 260 265 270
 Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr
 275 280 285
 Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr
 290 295 300
 Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln
 305 310 315 320
 Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg
 325 330 335
 Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly
 340 345 350
 Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly
 355 360 365
 Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile
 370 375 380
 Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly
 385 390 395 400
 Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr
 405 410 415
 Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr
 420 425 430
 Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu
 435 440 445
 Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu
 450 455 460
 Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr
 465 470 475 480
 Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp
 485 490 495

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Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys
 500 505 510
 Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met
 515 520 525
 Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu
 530 535 540
 Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg
 545 550 555 560
 Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala
 565 570 575
 Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His
 580 585 590
 Ala

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Gly Asp Lys Ile Met His Ala
 1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Thr Pro Trp Lys Val Leu Leu
 1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Trp Ser Tyr Gly
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Gly Xaa Ser Xaa Gly
1 5

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Other embodiments are within the following claims.

What is claimed is:

CLAIMS:

1. A nucleic acid encoding a polypeptide fragment of CD26 lacking amino acids 3-9 of intact CD26.
2. A nucleic acid encoding a polypeptide fragment of CD26 lacking amino acids 24-34 of intact CD26.
3. The nucleic acid of claim 1, wherein said polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2.
4. The nucleic acid of claim 2, wherein said polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 3.
5. A plasmid comprising the nucleic acid of any of claims 1 or 2.
6. A polypeptide fragment of CD26 capable of disrupting the naturally occurring binding interaction between CD45 and CD26.
7. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:
 - (a) providing a first and a second sample of cells expressing both CD26 and CD45;
 - (b) incubating said first sample in the presence of a candidate compound;
 - (c) incubating said second sample in the absence of said candidate compound;
 - (d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;
 - (e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

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(f) determining whether the amount of CD45 present in said first immunoprecipitate is less than the amount of CD45 present in said second immunoprecipitate, the presence of a lesser amount of CD45 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

8. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of CD26 present in said first immunoprecipitate is less than the amount of CD26 present in said second immunoprecipitate, the presence of a lesser amount of CD26 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

9. A monoclonal antibody which, when contacted under physiological conditions with a cell expressing CD26 and CD45, interferes with the association of said CD26 and CD45.

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10. A cell transfected with a nucleic acid encoding CD26, said cell expressing both CD26 and CD45 on its surface.

11. A cell transfected with a nucleic acid encoding CD45, said cell expressing both CD26 and CD45 on its surface.

12. A method of generating a hybridoma cell, said method comprising:

(a) providing a cell transfected with nucleic acid encoding CD26, such that said cell expresses CD26 on its surface;

(b) using said cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from said subject animal with a cell from an immortal cell line to produce a hybridoma cell.

13. A polypeptide fragment of CD26 capable of disrupting the naturally-occurring binding interaction between p43 and CD26.

14. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to p43, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and p43;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;

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(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of p43 present in said first immunoprecipitate is less than the amount of p43 present in said second immunoprecipitate, the presence of a lesser amount of p43 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

15. A polypeptide comprising the amino acid sequence of CD26 carboxy-terminal to Ala37, wherein at least one of the amino acids in the segment Gly627-Gly631 is deleted or replaced with a different amino acid.

16. A polypeptide fragment of CD26 lacking residues 1-34 of intact CD26.

17. A vaccine adjuvant comprising a fragment of CD26 in a pharmaceutically acceptable carrier.

18. A method of screening candidate immunosuppressive compounds, said method comprising:

(a) contacting a lymphocyte with CD26 or a fragment of CD26 in the presence of a candidate compound, and

(b) determining whether said candidate compound inhibits the CD26-enhanced proliferation of said lymphocyte, said inhibition being an indication that said candidate compound has immunosuppressive activity.

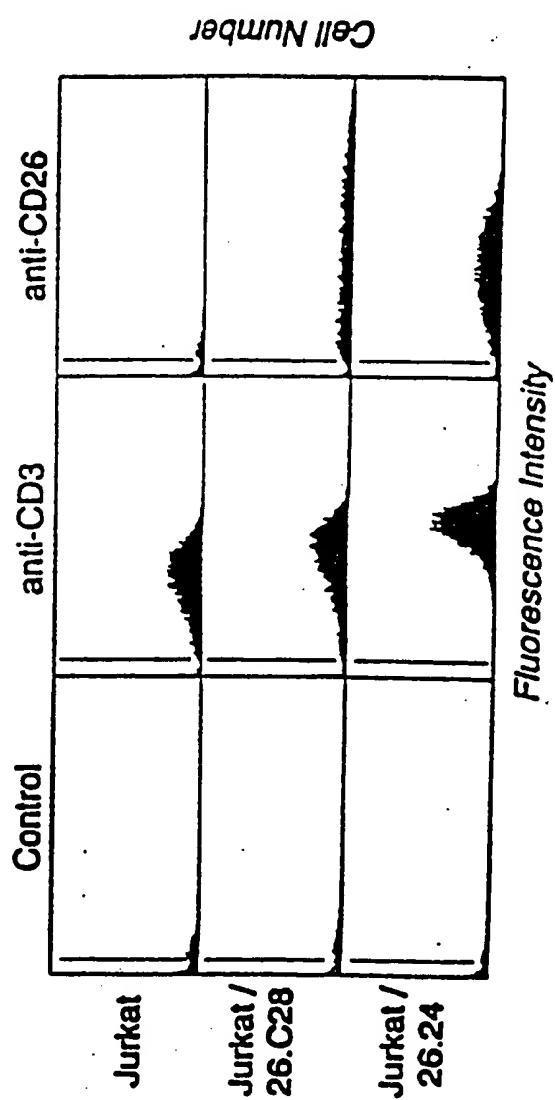
19. CD26 or a fragment thereof affixed to a solid matrix material.

FIG. 1

[illegible]

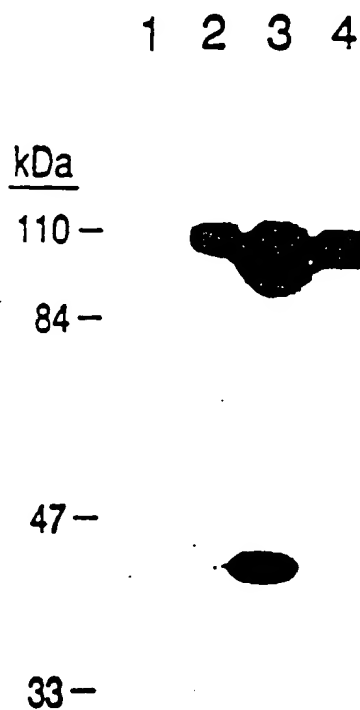
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FIG. 2



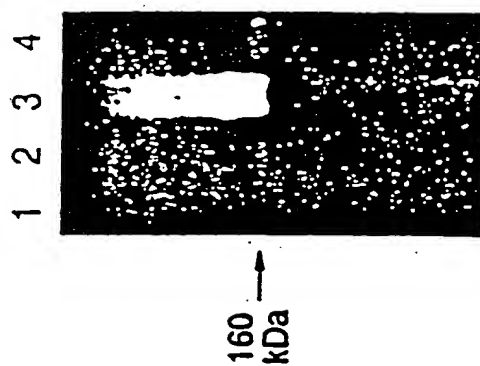
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FIG. 3A



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FIG. 3B



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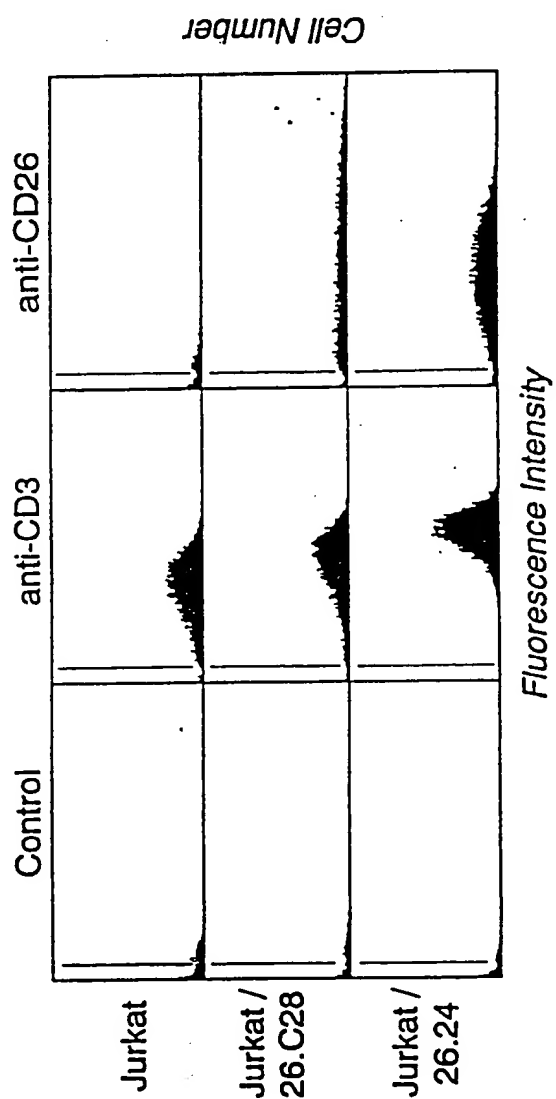


FIG. 2

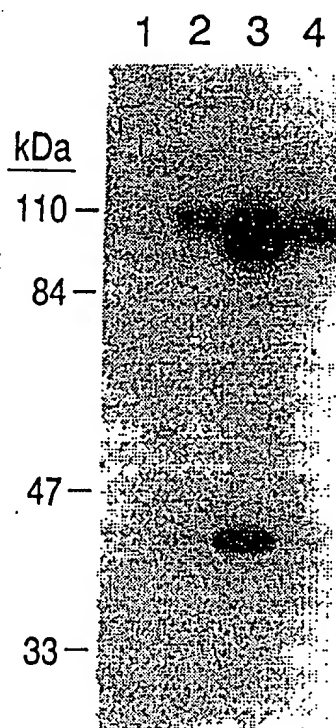


FIG. 3A

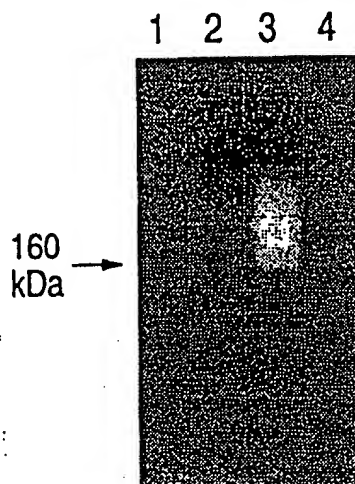


FIG. 3B

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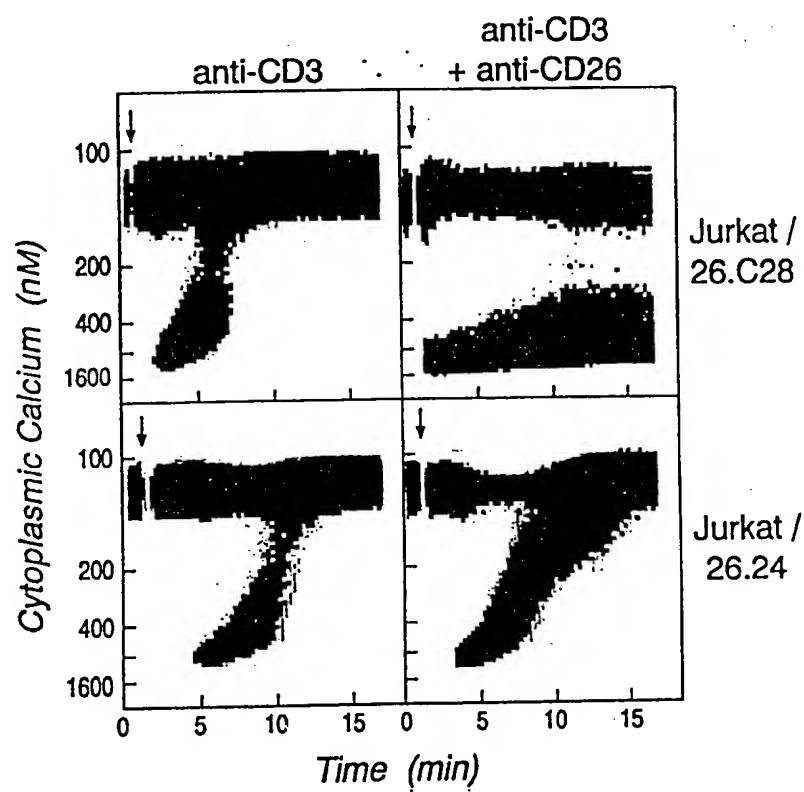


FIG. 4

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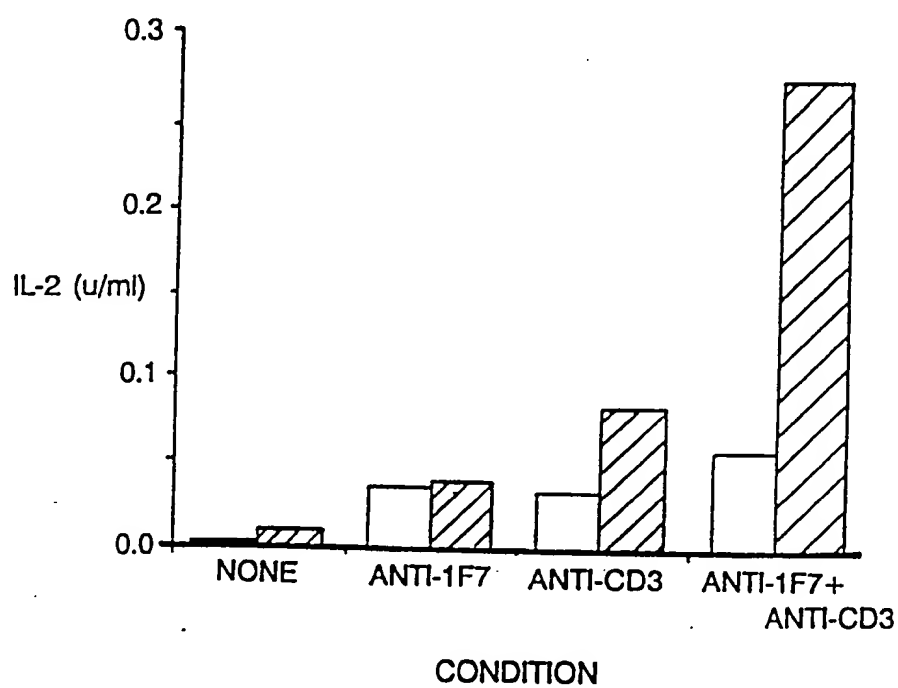


FIG. 5

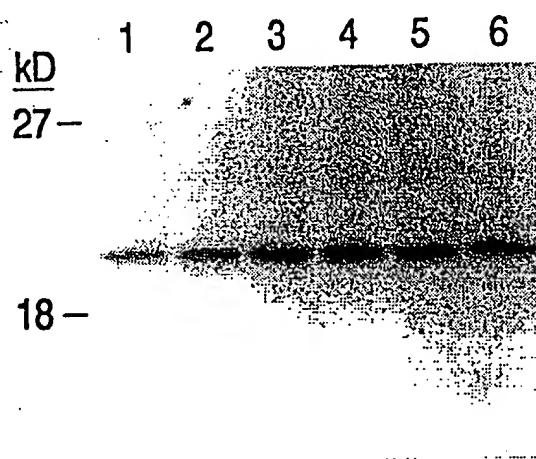


FIG. 6

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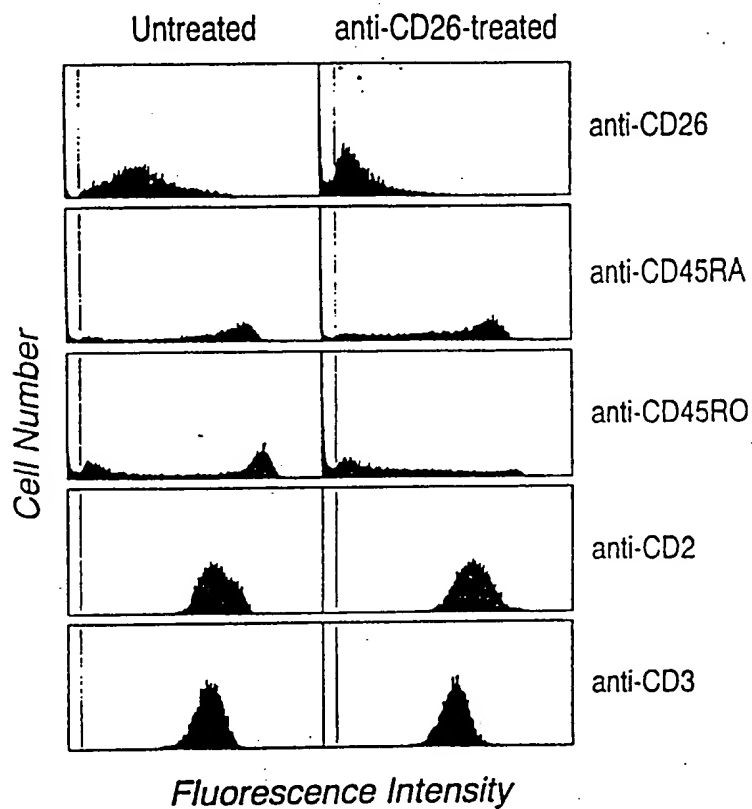


FIG. 7

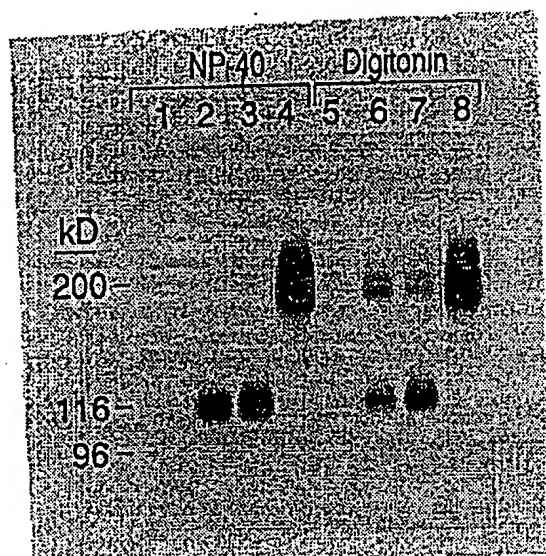


FIG. 8

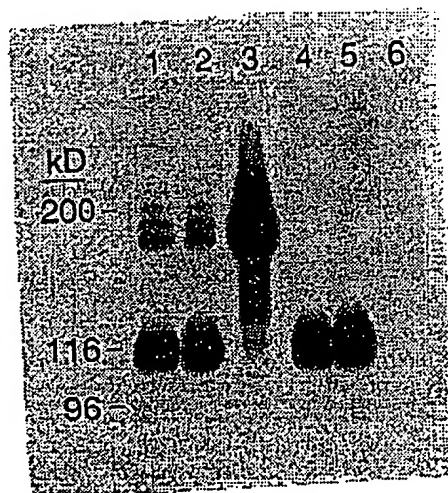


FIG. 9

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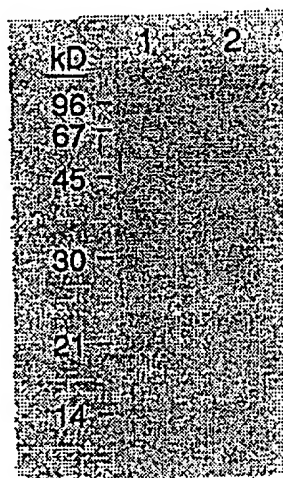


FIG. 10

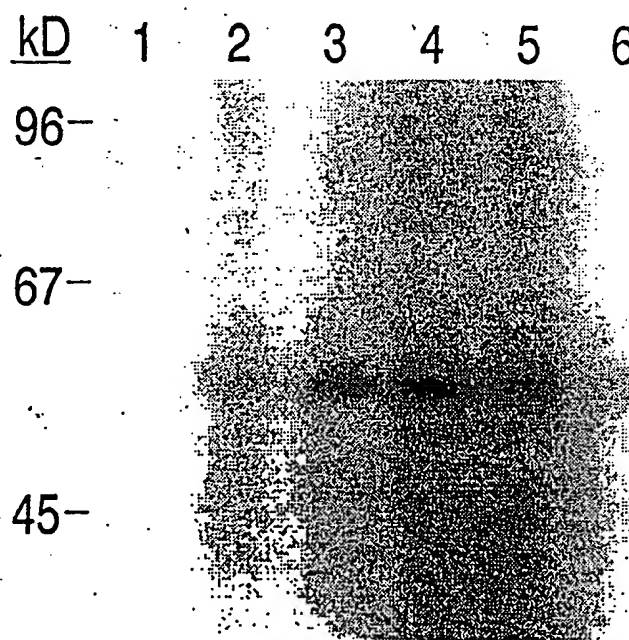


FIG. 11

SUBSTITUTE SHEET (RULE 26)

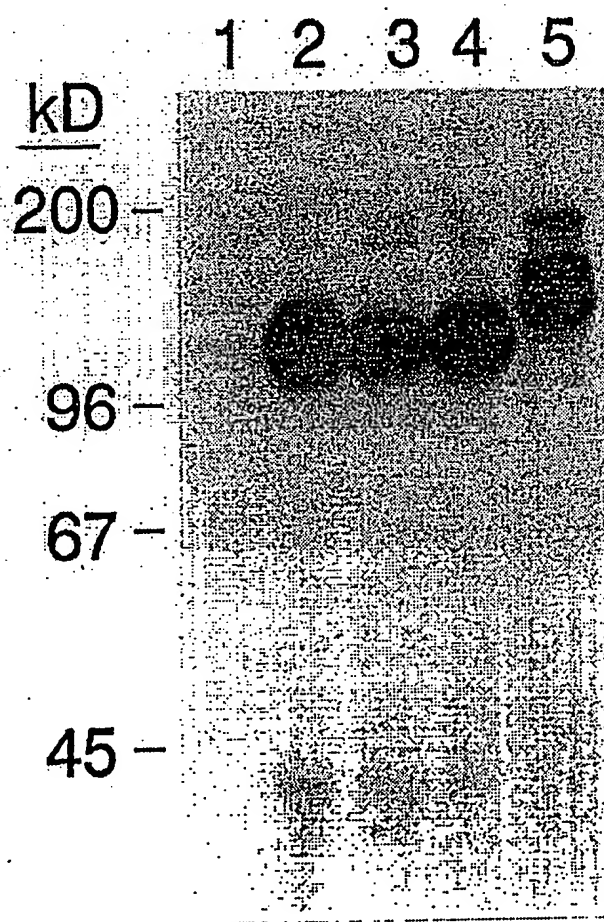


FIG. 12

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1
 CD26: MKTPWKVLLGLLGAALVTIITVPVLLNKGTDADSRKTYTLTDYLNKNTYRLKLYSLRWISDHEYLYKQENNI 51
 101
 LVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDDGQFILLEYN 151
 CD26: VKQWRHSYASYDIYDLNKRQLITEERIPNNTQVVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIYN 201
 GITDWVYEEVFSAYSALWSPNGTFLAYAQFNDTEVPLIEYSF 251
 CD26: YSDESLOQPKTVRPYPKAGAVNPTVKFFVNTDSLSSVTNATSIQITAPASMLIGDHLYLCDVTWATQERISLQWLR 301
 RIQYNSVMDICDYDESSGRWNCCLVARQHIEMSTTGWVGRFRPS 351
 CD26: EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYISNEYKGMPPGGRNLYKIQLS 401
 451
 DYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGL 501
 CD26: PLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPKSKLDFIILNETKFWYQMLPPHFDKSKKYPDLLDVYAGPCSQK 551
 ADTVFRLNWATYLASTENIIVASFEDGRSGYQGDKIMHAINRR 601
 CD26: LGTFEVEDQIEAARQFSKMGFVDNKRRIAIWGSYGGYVTSMLVLSGSGGVFKCGIAVAPVSRWEYDVSVYTERYMGLP 651
 701
 TPEDNLHDYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQS 751
 CD26 : AQISKALVDVGVDFAQMWYTDDEHGIASSTAQHIIYTHMSHFIIKQCFSLP

FIG. 13

1
 CD26: MKTPWKVLLGLGAAALVTIITVPVLLNKGTTDDATADSRKTYTLTDYLNKNTYRLKLYSLRWISDHEYLYKQENNI 51
 LVFNAEYGNSSVFLENSTFEFGHSINDYSISPDGQFILLEYN 101
 151
 CD26: VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNLPSYRITWTGKEDIYN 201
 GITDWWYEEVEFSAYSALWSPNGTFLAYAQFNDTEVPLIEYSF 251
 CD26: YSDESLQYPKTVRVPYPKAGAVNPTVKFFVNVNTDSLSSVTNATSIQITAPASMLIGDHYLCDVTWATQERISLQWLR 301
 RIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS 351
 CD26: EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISNEYKGMPPGGRNLYKIQLS 401
 451
 DYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSPGL 501
 CD26: PLYTLHSSVNDKGLRVLEDNSALDKMLQNVQMPKSKLDFFIILNETKFWYQMIILPHFDKSKKYPLLLDVYAGPCSQK 551
 ADTVFRLNWATYLASTENIIVASFDRGRSGYQGDKIMHAINRR 601
 CD26: LGTFEVEDQIEAARQFSKMGFVDNKRRIAWGWSYGGYVTSMLVSGSGVFKCGIAVAPVSRWEYDVSVYTERYMGLP 651
 701
 TPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQS 751
 CD26 : AQISKALVDVGVDVFQAMWYTDDEHGIASSTAHOHIYTHMSHFQKQCFSLP 751

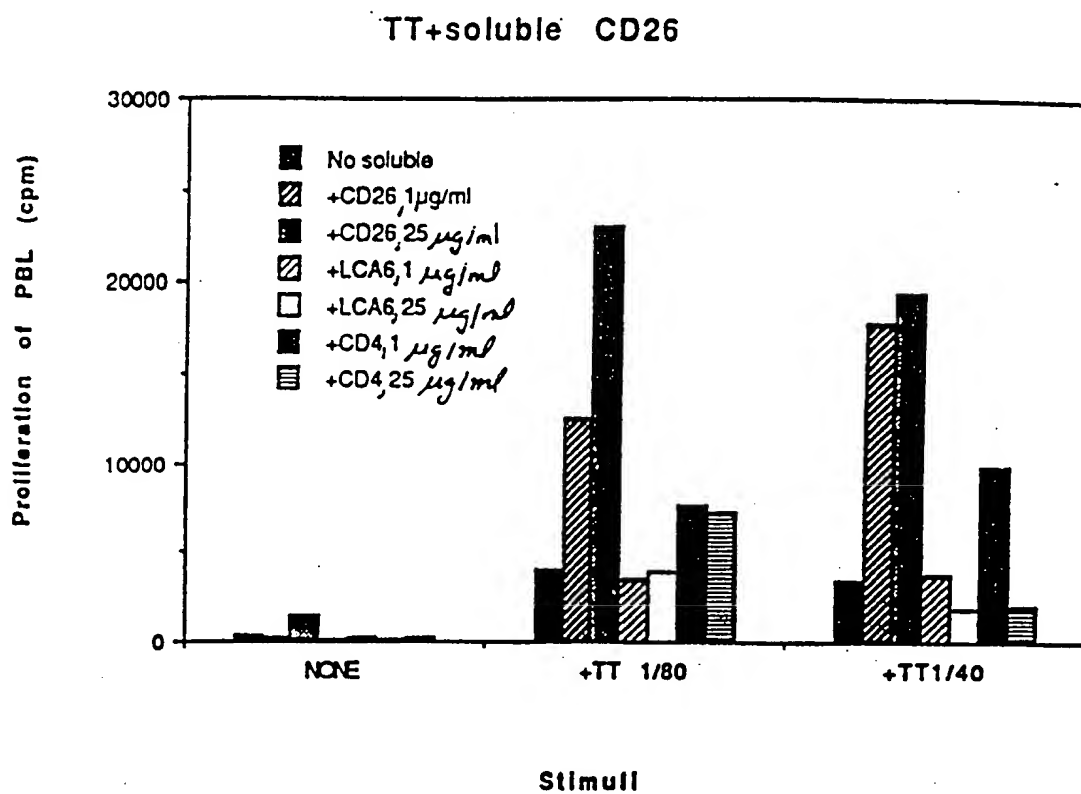
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1
CD26: MKTPWKVLLGLLGAALVTIITVPVLLNKGTTDADSRKTYTLTDYLNKNTYRLKLYSL 51
RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYN 101
151
VKQWRHSYASYDIYDLNKRQLITEERIPNNTQVWTSVPVGHKLAYVWNNDIYVKIEPNL
201
PSYRITWTGKEDIINYGITDWWYEEVFSAVSALWSPNGTFLAYAQENDTEVPLIEYSF
251
YSDSLQYPKTVRVPYPKAGAVNPTVKFFVNTDSLSSVTNATSIQITAPASMLIGDHYL
301
CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS 351
EPHFTLDGNSFYKIIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSYLYYISN 401
451
EYKGMPPGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGPLPY
501
TLHSSVNDKGLRVLEDNSALDKMLQNVQMPKLLDFIILNETKFWYQMILPPHFDKSKKY
551
PLLLDVYAGPCSQKADTVFRLNWTATYLASTENIIVASEDGRSGYQGDKIMHA

FIG. 15

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FIG. 16



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07923

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 435/320.1, 240.2, 240.27, 69.1, 7.24; 530/395, 388.7, 402; 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Volume 264, Number 6, issued 25 February 1989, S. Ogata <i>et al.</i> , "Primary Structure of Rat Liver Dipeptidyl Peptidase IV Deduced from Its cDNA and Identification of the NH ₂ -terminal Signal Sequence as the Membrane-anchoring Domain", pages 3596-3601, especially the abstract.	1-19
Y	Biochimica et Biophysica Acta, Volume 1131, issued 1992, Y. Misumi <i>et al.</i> , "Molecular cloning and sequence analysis of human dipeptidyl peptidase IV, a serine proteinase on the cell surface", pages 333-336, especially the abstract.	1-8, 11-19

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

05 November 1993

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/07923

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Volume 263, Number 32, issued 15 November 1988, S. R. Schmid <i>et al.</i> , "Deletion of the Amino-terminal Domain of Asialoglycoprotein Receptor H1 Allows Cleavage of the Internal Signal Sequence", pages 16886-16891, especially the abstract.	15-19
Y	Proceedings of the National Academy of Sciences of the USA, Volume 84, issued December 1987, A. Aruffo <i>et al.</i> , "Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system", pages 8573-8577, especially the abstract.	1-19
Y	Methods in Enzymology, Volume 152, issued 1987, W. I. Wood, "Gene Cloning Based on Long Oligonucleotide Probes", pages 443-447, see the entire document.	1-19
A	Molecular Immunology, Volume 29, Number 2, issued February 1992, Y. Torimoto <i>et al.</i> , "Biochemical characterization of CD26 (dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies", pages 183-192.	1-19
A	Biochemistry, Volume 28, Number 21, issued 1989, W. Hong <i>et al.</i> , "Expression of Enzymatically Active Rat Dipeptidyl Peptidase IV in Chinese Hamster Ovary Cells after Transfection", pages 8474-8479.	1-19
A	Scandinavian Journal of Immunology, Volume 31, Number 4, issued April 1990, A. J. Ulmer <i>et al.</i> , "CD26 Antigen is a Surface Dipeptidyl Peptidase IV (DPPIV) as Characterized by Monoclonal Antibodies Clone TII-19-4-7 and 4EL1C7", pages 429-435.	1-19

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US93/07923

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Volume 267, Number 7, issued 05 March 1992, D. Darmoul <i>et al.</i> , "Dipeptidyl Peptidase IV (CD 26) Gene Expression in Enterocyte-like Colon Cancer Cell Lines HT-29 and Caco-2: cloning of the complete human coding sequence and changes of dipeptidyl peptidase IV mRNA levels during cell differentiation", pages 4824-4833, especially the abstract.	1-19
Y	Journal of Immunology, Volume 147, Number 8, issued 15 October 1991, Y. Torimoto <i>et al.</i> , "Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes", pages 2514-2517, especially the abstract and page 2517.	7-11
X	Journal of Immunology, Volume 141, Number 11, issued 01 December 1988, M. Streuli <i>et al.</i> , "Characterization of CD45 and CD45R monoclonal antibodies using transfected mouse cell lines that express individual human leukocyte common antigens", pages 3910-3913, especially the abstract.	9
X	Journal of Immunology, Volume 149, Number 4, issued 15 August 1992, G. A. Koretzky <i>et al.</i> , "Restoration of T cell receptor-mediated signal transduction by transfection of CD45 cDNA into a CD45-deficient variant of the Jurkat T cell line", pages 1138-1142, especially the abstract.	11
Y	Journal of Cell Biology, Volume 111, issued August 1990, W. Hong <i>et al.</i> , "Molecular Dissection of the NH ₂ -Terminal Signal/Anchor Sequence of Rat Dipeptidyl Peptidase IV", pages 323-328, especially the abstract.	13, 15-19
Y,P	Science, Volume 261, issued 23 July 1993, J. Kameoka <i>et al.</i> , "Direct Association of Adenosine Deaminase with a T Cell Activation Antigen, CD26", pages 466-469, especially the abstract.	13, 14
Y	Nature, Volume 334, issued 11 August 1988, S. Brenner, "The molecular evolution of genes and proteins: a tale of two serines", pages 528-530, especially the abstract and Table I.	15
Y	Journal of Biological Chemistry, Volume 266, Number 15, issued 25 May 1991, G. Pei <i>et al.</i> , "Expression, Isolation, and Characterization of an Active Site (Serine-528--> Alanine) Mutant of Recombinant Bovine Prothrombin", pages 9598-9604, especially the abstract.	15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/07923

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C12N 15/12, 15/10, 15/85, 5/10, 5/12; C07K 13/00, 17/02, 15/28; A61K 37/02

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/23.2; 435/320.1, 240.2, 240.27, 69.1, 7.24; 530/395, 388.7, 402; 514/2, 12

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Text databases: Medline, Biosis, SciSearch, CAS, Embase, USPTO-APS

Search terms: CD26, thymocyte activating molecule, dipeptidyl peptidase IV; CD45, leukocyte common antigen; clon?, recombinant, cDNA, mRNA, PCR, transfect?

Sequence databases: GenBank, EMBL, GeneSeq, SwissProt, PIR